CHA Doc. No. 77ACP

VOLUME _____ OF _____ OF SUBMISSION

ACEPHATE, AZINPHOS-METHYL, CHLORPYRIFOS, DIAZINON, MALATHION AND METHAMIDOPHOS, AZINPHOS-METHYL OXON, CHLORPYRIFOS OXON, DIAZINON OXON, MALATHION OXON: FINAL REPORT

TITLE

Chlorine Degradation of Six Organophosphorus Insecticides and Four Oxons in a Drinking Water Matrix

DATA REQUIREMENT

Not Applicable

AUTHORS

Dennis P. Tierney, Brian R. Christensen, Victor C. Culpepper

COMPLETION DATE

July 15, 2000

PERFORMING COMPANIES

Syngenta Crop Protection, Inc.
410 Swing Road
Greensboro, NC 27409

En-fate, LLC
14280 23rd Ave N
Plymouth, MN 55447

EASI Laboratory
2501 Lexington Ave.
Kenner, LA 70062

LABORATORY STUDY IDENTIFICATION

Syngenta Study No. 1562-00 *En fate* Study No. 00102

SUBMITTER/SPONSORS

Cholinesterase Risk Assessment Case Study Team

VOLUME 1 OF 1 OF STUDY

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d) (1) (A), (B), or (C).

Company:	Syngenta Crop Protection, Inc.
Company Representative:	Carolyn F. Brinkley
Title:	Senior Regulatory Manager

Carolyn F Brinkley
Signature

June 25, 2001
Date:

These data are the property of Syngenta Crop Protection, Inc., Bayer Corporation, Dow Agrosciences LLC, Cheminova Agro A/S, and Valent U.S.A. and as such, are considered confidential for all purposes other than compliance with FIFRA §10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality, which may exist under any other statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study was conducted according to Good Laboratory Practice (GLP) requirements of 40 CFR Part 160, EPA FIFRA. A laboratory audit was conducted by Syngenta Crop Protection, Inc. prior to the commencement of the project.

Two protocol amendments were approved. One protocol deviation is reported.

6-20-2001

Dennis P. Tierney, Ph.D. Date
Study Director/Submitter/Sponsor,
Chloinesterase Risk Assessment Case Study Team

REPORT APPROVAL

TITLE: CHLORINE DEGRADATION OF SIX ORGANOPHOSPHORUS INSECTICIDES AND FOUR OXONSIN A DRINKING WATER MATRIX

Syngenta Study No.: 1562-00

En.fate Study No.: 00102

Experimental Start Date: April 1, 1999

Experimental Termination Date: July 15, 2000

STUDY DIRECTOR, Syngenta Crop Protection, Inc.

6-20-2001

Dennis P. Tierney, Ph.D.
Environmental Product Manager
Environmental Stewardship and Regulatory Policy

Study Coordinator, *En.fate, LLC*

6.19.01

Brian R. Christensen Date
Principal Scientist

Management, Syngenta Crop Protection, Inc.

6/20/01

Janis McFarland, Ph.D. Date
Director
Environmental Stewardship and Regulatory Policy

Quality Assurance, Syngenta Crop Protection, Inc.

6/25/01

Tom Gale, Jr. Date
Manager
Quality Assurance Unit

PRINCIPAL STUDY PERSONNEL

Study Director

Dennis Tierney
Syngenta Crop Protection, Inc.
410 Swing Road
Greensboro, NC 27409
Tel: 336-632-2850
Fax: 336-632-2997

Study Coordinator

Brian R. Christensen
En fate, LLC
14280A 23rd Ave N
Plymouth, MN 55447
Tel: (612) 559-9101
Fax: (612) 559-9184

Laboratory Coordinator

Victor C. Culpepper Sc.D.
EASI, Laboratory
2501 Lexington Ave.
Kenner, Louisiana 70062
Tel: (540)-469-3685
Fax: (504)-469-0140

Acknowledgement

The principal study personnel would like to acknowledge and thank the following individuals for their assistance and direction in designing and performing the study and reviewing the final reports for this project:

Nicholas Poletika, Ph.D. Dow AgroSciences,
David Wustner, Ph.D. Valent U.S.A.
Pete Coody Ph.D. and John Fisher, Bayer Corporation
Paul Whatling, Cheminova, Inc.
Robert Butz Ph.D. and Matt Brooks, Jellinek Schwartz and Connolly, Inc.
Rick Riess, Sciences International

QUALITY ASSURANCE STATEMENT

Study Title: CHLORINE DEGRADATION OF SIX ORGANOPHOSPHORUS INSECTICIDES AND FOUR OXONS IN A DRINKING WATER MATRIX

Study Director: Dennis Tierney

Study No.: Syngenta 1562-00 ; En-fate, LLC 00102

Pursuant to Good Laboratory Practice Standards, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to the Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

INSPECTION/ AUDIT TYPE	INSPECTION AUDIT DATE	REPORTING DATES	PERFORMED BY
GLP Facility Inspection	1/28/99	3/23/99	Novartis QAU
Protocol Audit	5/10-18/99	5/21/99	Novartis QAU
In-progress Inspection	6/30-7/1/99	7/23/99	Novartis QAU
Raw Data and Final Report Audit	6/1/00-7/1/00	7/15/00	EASI QAC
Final Report Audit (No Raw Data)	7/15/00-6/7/01	6/7/01	Syngenta QAU

Prepared by: Renee Tetlow Date 6/14/01

Renee Tetlow, EASI
Quality Assurance Coordinator

TABLE OF CONTENTS

Section **Page No.**

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

EXECUTIVE SUMMARY

This study evaluates the degradation of six organophosphorus insecticides and four of their oxons (hereinafter degradates) by chlorine in finished drinking water. This study was conducted in conjunction with the OP Monitoring Program.

Chlorine is used as a disinfectant in all the water treatment plants participating in the OP Monitoring Program. Chlorine may have an oxidative or other degradative effect on OP insecticides and their oxons. This may influence their occurrence in finished drinking water samples. The objective of this study was to evaluate the effect of total residual chlorine on the integrity of six organophosphorus insecticides and four of their degradates at typical chlorine concentrations and contact time intervals seen at water treatment plants. Total residual chlorine is defined as free and combined chlorine, existing as hypochlorous acid and hypochlorite ion (free chlorine) and monochloramine, and, to a lesser extent, di- and trichloramine (residual chlorine).

Insecticide and oxon degradation was evaluated in finished drinking water at chlorine concentrations of 2 mg/L and 4 mg/L compared to degradation in finished water containing no chlorine at time intervals up to 24 hours. Chlorpyrifos, diazinon, azinphos-methyl and malathion samples were extracted using Baker Corporation C-18 solid phase extraction disks and analyzed by gas chromatography/mass spectrometry in selected ion mode. Acephate and methamidophos samples were extracted using Waters Corporation AC-2, graphitized carbon, solid phase extraction tubes and analyzed by gas chromatography/flame photometric detection.

Chlorine (sodium hypochlorite) at an initial concentration of 2 mg/L exerts an oxidative effect on the parent insecticides. These effects range from 47 to 53% decrease in parent insecticide present within fifteen minutes. The oxidative effects are essentially complete within 15 minutes with the exception of acephate (60 minutes) and methamidophos (24 hours). Insecticide oxon formation occurred during parent degradation.

There was degradation of diazinon oxon, malathion oxon and azinphos methyl oxon at a chlorine concentration 2mg/L. Chlorpyrifos oxon was degraded by 9%.

Chlorine (sodium hypochlorite) at an initial concentration of 4 mg/L completely degrades diazinon, malathion, azinphos methyl, and acephate within 15 minutes of contact time. Chlorpyrifos is 94% degraded within 15 minutes and 100% within 30 minutes. Methamidophos degradation was 69% complete after 15 minutes, 95% after 60 minutes and 100% by 24 hours of contact.

There was no degradation of malathion oxon or azinphos methyl oxon over 24 hours. Diazinon oxon and chlorpyrifos oxon were degraded 22% and 25% respectively over 24 hours.

All oxons are significantly more resistant to chlorine degradation compared to the parent compounds. However, the oxons are more susceptible to degradation during 24-hour storage at ambient temperature than the parent compounds, particularly for malathion oxon and azinphos methyl oxon. A process other than oxidation is causing partial degradation of the insecticide oxons.

INTRODUCTION

Chlorine is the most commonly used chemical for disinfection of raw and finished drinking water in community water treatment plants. Chlorine may be added in the front of the water treatment train (initial) to destroy living organisms and assist in breaking down complex compounds. Chlorine may also be added after the flocculation/sedimentation treatment processes. Chlorine (or chloramines) is added to the newly treated water as it is being discharged into the storage/distribution system. In many distribution systems, a "boost" of chlorine is added to ensure that a detectable level of chlorine is present at all points of the system. This study evaluates the degradation of six organophosphorus insecticides and four of their oxons by chlorine in finished drinking water. The target insecticides and oxons (in parentheses) include: acephate, methamidophos, diazinon (diazinon oxon), chlorpyrifos (chlorpyrifos oxon), malathion (malathion oxon), and azinphos methyl (azinphos methyl oxon). Methamidophos is also a non-oxidative degradation product of acephate.

Insecticide and oxon degradation was evaluated in finished drinking water at chlorine concentrations of 2 mg/L and 4 mg/L compared to degradation/hydrolysis in finished water containing no chlorine at time intervals up to 24 hours. The chlorine concentrations and the 24-hour time frame represent typical chlorine target concentrations and finished water storage at water treatment plants participating in the OP Monitoring Program (Drinking Water Monitoring Study for Six Organophosphate Insecticides and Four of Their Oxon Degradation Products from 44 Community Water Systems on Surface Water in the United States - Table 5-5).

STUDY OBJECTIVE

This study was completed in conjunction with the OP Monitoring Program cited above. Chlorine is used as a disinfectant in every water treatment plant participating in the OP Monitoring Program. Chlorine may have an oxidative or other degradative effect on OP insecticides and their oxons. This may influence their occurrence in finished drinking water samples. The objective of this study was to evaluate the effect of total residual chlorine on the integrity of six organophosphorus insecticides and four of their oxons at typical chlorine concentrations and contact time intervals seen at water treatment plants.

TEST SITE

Laboratory experimentation was performed at Environmental Analytical Solutions, Inc., 2501 Lexington Ave, Kenner, Louisiana 70062.

EXPERIMENTAL METHODS

Phase 1: Evaluation of diazinon, diazinon oxon, chlorpyrifos, chlorpyrifos oxon, malathion, malathion oxon, azinphos methyl, and azinphos methyl oxon

Two separate chlorine degradation studies were performed; one degradation study evaluated the organophosphorus insecticides and one degradation study evaluated organophosphorus insecticide oxons. This was performed due to the potential degradation of the insecticides into their oxons which may mask the degradation of the oxons over time.

Preparation of Insecticide Standards

A working standard containing diazinon, chlorpyrifos, malathion, and azinphos methyl was prepared at a concentration of 2.00 mg/l for each compound by diluting stock standards in acetone to a final volume of 100 ml in a class A volumetric flask:

Stock Standard	Stock Conc., mg/l	Volume Used, ul	Working Standard Conc., mg/l
Diazinon	16,800	11.9	2.00
Chlorpyrifos	9,700	20.6	2.00
Malathion	15,300	13.1	2.00
Azinphos methyl	1,820	110	2.00

A separate working standard containing diazinon oxon, chlorpyrifos oxon, malathion oxon, and azinphos methyl oxon was prepared at a concentration of 2.00 mg/l for each compound by diluting stock standards in acetone to a final volume of 100 ml in a class A volumetric flask:

Stock Standard	Stock Conc., mg/l	Volume Used, ul	Working Standard Conc., mg/l
Diazinon oxon	17,100	11.7	2.00
Chlorpyrifos oxon	9,350	21.4	2.00
Malathion oxon	13,600	14.7	2.00
Azinphos methyl oxon	11,000	18.2	2.00

Preparation of Chlorinated Finished Drinking Water

Two 50-liter, HDPE carboys were filled with laboratory finished drinking water and allowed to sit for 24 hours in order to reduce the concentration of total residual chlorine prior to performing the chlorination study. After 24 hours, the total residual chlorine of the finished drinking water was determined using a DPD colorimetric procedure and color measurement using Hach DR100 Colorimeter. The total residual chlorine

concentration was 0.02 mg/l as Cl_2. Due to the low level of chlorine present, the chlorine was not quenched prior to spiking the water with a sodium hypochlorite solution.

The total residual chlorine concentration of a stock standard (approximate 5.25% solution) was determined using the DPD colorimetric method by diluting 100 ul of the stock standard in 1 liter of water using an eppendorf micropipette and a class A volumetric flask. The actual concentration was determined to be 31,000 mg/l.

Two 20-liter aliquots of the finished drinking water were added to 50-liter HDPE carboys using a class A graduated cylinder. The carboys were fortified with 2.00 mg/l and 4.00 mg/l of sodium hypochlorite by adding 1.29 ml and 2.58 ml respectively of the stock standard to the water using an adjustable eppendorf pipette. The carboys were vigorously mixed for approximately 1 minute and then analyzed for total residual chlorine using the DPD colorimetric method. The actual concentrations were determined to be 1.9 mg/L and 4.1 mg/L.

For the preparation of the control samples, a 10-liter aliquot of the finished drinking water was added to a 20-liter HDPE carboy and fortified with 2.00 mg/l of sodium hypochlorite by adding 0.65 ml of the stock standard to the water. The carboy was mixed for 1 minute and the hypochlorite was quenched by adding 3.0 grams of sodium thiosulfate to the water and mixing vigorously for 2 minutes. A chlorine analysis of the quenched water determined that all of the residual chlorine was removed by the sodium thiosulfate.

Preparation of Insecticide Fortified Water

The two 20-liter aliquots of water were fortified with the parent insecticide mixture by adding 5.00 ml of the 2.00 mg/l working standard to each carboy using a Hamilton precision syringe, providing an initial concentration of 0.500 ug/l for each insecticide. The 10-liter aliquot (control) was fortified by adding 2.5 ml of the working standard to the water. The carboys were immediately mixed for 30 seconds and a timer was set to initiate the timing sequence. The spiked water was equally partitioned into 1-liter amber, borosilicate glass jars to provide the following experimental matrix:

	Cl_2 Concentration, mg/l		
Time	**0**	**2.0**	**4.0**
0 min.	3 reps		
15 min.		3 reps	3 reps
30 min.		3 reps	3 reps
60 min.		3 reps	3 reps
24 hrs.	3 reps	3 reps	3 reps

After each contact time, the sodium hypochlorite was removed by adding a small scoop (app. 300 mg) of sodium thiosulfate to each bottle and mixing thoroughly. Complete residual chlorine removal was verified by analyzing total residual chlorine in one

replicate fortified at 4.0 mg/l chlorine. After chlorine removal, the replicates were refrigerated at 4°C prior to extraction.

The same insecticide fortified water procedure and experimental matrix was conducted for the insecticide oxons exactly as described above for the parent insecticide compounds.

Sample Extraction, Analysis, Method Detection Limits

Samples were extracted using Baker Corporation C-18 solid phase extraction disks and analyzed by gas chromatography/mass spectrometry in selected ion mode. Extraction was performed according to the laboratory standard operating procedure EASI SOP MS-20.03 (Appendix B Protocol and Amendments).

Analysis of C-18 extracts for azinphos-methyl, chlorpyrifos, diazinon, malathion, their four oxons and associated quality control samples were conducted according to the laboratory standard operating procedure EASI SOP MS-20.03 (Appendix A). Samples were analyzed using a Hewlett Packard Model 5890 Series II, Gas Chromatograph and Model 5971 Mass Selective Dectector operating in selective-ion mode. A 15 m x 0.25 mm inside diameter Restek RTX 200 column containing a 1-um crossbond, trifluoropropylmethyl polysiloxane film was utilized for analyte separation and resolution.

A method detection limit (MDL) study was performed by extracting and analyzing 15 replicates of finished drinking water fortified with the target analytes at a concentration of 0.050 ug/l. The MDL was calculated by multiplying the standard deviation of the replicates by the student's *t* value for the number of replicates with n-1 degrees of freedom. The calculated MDL and practical quantitation limit (PQL) values for each MDL study are shown in Table 1. The PQL is defined as five times the MDL, or 0.050 ug/l whichever is greater. Sample results greater than the PQL were reported as detected values. Analytes detected below the PQL were quantified and reported if the chromatography and qualification (GC/MS) provided reasonable justification for their inclusion. All values reported below the PQL should be considered non-detections.

Phase 2: Evaluation of acephate and methamidophos

Preparation of Insecticide Standards

An intermediate, 100 mg/l stock standard of acephate was prepared by diluting 185 ul of a 5,400 mg/l stock standard in 10.0 ml of acetone in a 10 ml class A volumetric flask. A 2.00-mg/L acephate working standard was prepared by diluting 200 ul of the intermediate standard in 10 ml of acetone in a class A volumetric flask.

An intermediate, 100 mg/l stock standard of methamidophos was prepared by diluting 189 ul of a 5,300 mg/l stock standard in 10.0 ml of acetone in a 10 ml class A volumetric flask. A 2.00-mg/l methamidophos working standard was prepared by diluting 200 ul of the intermediate standard in 10 ml of acetone in a class A volumetric flask.

Stock Standard	Stock Conc., mg/l	Volume Used, ul	Working Standard Conc., mg/l
Acephate	5,400	185	2.00
Methamidophos	5,300	189	2.00

Preparation of Chlorinated Finished Drinking Water

Preparation of chlorinated finished drinking water was prepared exactly as described above (page 10). After the 24-hour residence time the residual chlorine remaining in the finished drinking water was 0.05 mg/l. A new sodium hypochlorite standard was purchased for use and the total residual chlorine of the stock standard was determined using the DPD colorimetric method as described previously and the concentration was determined to be 36,000 mg/l.

Two 20-liter aliquots of the finished drinking water were added to 50-liter HDPE carboys using a class A graduated cylinder. The carboys were fortified with 2.00 mg/l and 4.00 mg/l of sodium hypochlorite by adding 1.08 ml and 2.19 ml respectively of the stock standard to the water using an adjustable eppendorf pipette. The carboys were vigorously mixed for approximately 1 minute and then analyzed for total residual chlorine using the DPD colorimetric method. The actual concentrations were determined to be 2.0 mg/L and 4.1 mg/L.

For the preparation of the control samples, a 10-liter aliquot of the finished drinking water was added to a 20-liter HDPE carboy and fortified with 2.00 mg/l of sodium hypochlorite by adding 0.54 ml of the stock standard to the water. The carboy was mixed for 1 minute and the hypochlorite was quenched by adding 3.0 grams of sodium thiosulfate to the water and mixing vigorously for 2 minutes. A chlorine analysis of the quenched water determined that all of the residual chlorine was removed by the sodium thiosulfate.

Preparation of Insecticide Fortified Water

A 5.00 ml aliquot of the 2.00 mg/l acephate standard was added to each of two 500-ml, borosilicate glass flasks using a Hamilton precision syringe. The acetone solvent was allowed to evaporate under a vacuum hood, and the acephate residues were reconstituted in 200 ml of deionized water by vigorously mixing the flasks for 3 minutes.

One reconstituted standard was added to each carboy containing the 20 liters of chlorinated finished drinking water.

The above procedure was repeated for the control finished drinking water by adding 2.5 ml of the acephate standard to a 500 ml flask, evaporating and reconstituting as described above, and adding to the 10-liter portion of dechlorinated finished drinking water.

The three carboys containing the fortified finished drinking water were vigorously mixed for 60 seconds and a timer was set to initiate the timing sequence. The water was

partitioned into 1-liter amber borosilicate glass jars, quenched, and divided into replicates exactly as described in the Phase 1 experimental procedures (page 11). All replicates were stored at 4°C after the appropriate contact time prior to sample extraction.

The same insecticide fortified water procedure and experimental matrix was conducted for methamidophos exactly as described above for acephate.

Sample Extraction and Analysis

Samples were extracted using Waters Corporation AC-2, graphitized carbon, solid phase extraction tubes and analyzed by gas chromatography/flame photometric detection. Extraction was performed according to the laboratory standard operating procedure EASI SOP MS-20.04 (Appendix B, Protocol).Analysis of AC-2 extracts for acephate and methamidophos and associated quality control samples were analyzed according to the laboratory standard operating procedure EASI SOP MS-20.04 (Appendix B). Samples were analyzed using a Hewlett Packard Model 5890 Series II, Gas Chromatograph equipped with a flame photometric detector. A 30 m x 0.53 mm i.d. Restek RTX 200 column containing a 1-μm crossbond, trifluoropropylmethyl polysiloxane film was utilized for analyte separation and resolution.

A method detection limit (MDL) study was performed by extracting and analyzing 15 replicates of finished drinking water fortified with the target analytes at a concentration of 0.050 ug/l. The MDL was calculated by multiplying the standard deviation of the replicates by the student's *t* value for the number of replicates with n-1 degrees of freedom. The calculated MDL and practical quantitation limit (PQL) values for each MDL study are shown in Table 1. The PQL is defined as five times the MDL, or 0.050 ug/l whichever is greater. Sample results greater than the PQL were reported as detected values. Analytes detected below the PQL were quantified and reported if the chromatography and qualification (GC/MS) provided reasonable justification for their inclusion. All values reported below the PQL should be considered non-detections.

QUALITY CONTROL, QUALITY ASSURANCE, STATISTICAL METHODS

Quality Control, Method and Matrix Spikes

All samples were extracted as a "sample set". A sample set is defined as a group of 20 samples extracted at one time with associated quality assurance samples. Each sample set consisted of the experimental samples, a method blank, a matrix blank, a matrix spike and a matrix spike duplicate. A method blank consists of laboratory-deionized water extracted and analyzed exactly as the time interval samples. A matrix blank consists of a laboratory potable water sample extracted and analyzed exactly as the samples. The matrix spike and matrix spike duplicate samples consist of the same laboratory potable water as the matrix blank, fortified with the target analytes and extracted and analyzed exactly as the time interval samples. Matrix spikes were prepared using laboratory potable water. Matrix spikes were fortified with the target analytes to provide a final concentration of 0.500 μg/L of each analyte.

Quality Assurance

EASI was audited by Syngenta Crop Protection Quality Assurance Unit (Syngenta QAU) prior to the commencement of the analytical work. Syngenta QAU also performed an in-progress inspection of the laboratory. This report was submitted to Syngenta QAU for auditing.

Statistical Methods

The degradation of target analytes in the experimental samples were compared to the control samples using a two-sample *t* test for independent variables with equal variances (t-sided) at the 95% confidence interval.

Data Storage

All raw data, the protocol, protocol amendments, study deviations and the project report are archived in the Syngenta Agricultural Group Archives of Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC, 27409.

RESULTS AND DISCUSSION

Phase 1: Evaluation of diazinon, diazinon oxon, chlorpyrifos, chlorpyrifos oxon, malathion, malathion oxon, azinphos methyl, and azinphos methyl oxon

Evaluation of Compound Stability in Control Samples

Control samples were utilized to evaluate the significance of compound degradation resulting from the 24-hour storage time period. It is important to note that the evaluation of compound degradation in the presence of chlorine was performed by comparison against the time equals zero control samples. As was expected, and confirmed by the control samples, compound loss is negligible during the first 60 minutes of storage. In evaluating compound loss after 1 hour, the reduction may be attributed primarily to chlorine oxidation and partially to hydrolysis.

The stability of the target analytes were evaluated in the control samples over a 24-hour time interval using a two-sample *t* test for independent variables with equal variances (t-sided) at the 95% confidence interval. Diazinon, chlorpyrifos, and azinphos methyl were stable as they did not significantly degrade over 24-hours (Figure 1). Diazinon degraded 3%, chlorpyrifos 4%, and azinphos methyl 10%. Malathion loss was significant; a 24% reduction was noted after 24 hours. No formation of oxons was noted in the 24-hour control samples.

Significant degradation of all oxon controls occurred during the 24 hour time period: Diazinon oxon was reduced by 10%, chlorpyrifos oxon by 15%, malathion oxon by 45% and azinphos methyl oxon 38% (Figure 2).

Evaluation of Compound Degradation at 2 mg/L Chlorine Concentration

Parent insecticide degradation (and resultant oxon formation) and oxon degradation at each time interval at a 2 mg/L chlorine concentration are shown in Tables 2 and 3, and Figures 3 through 12.

Partial degradation of all parent insecticides occurred. The entire degradation occurred within the first sampling (15 minutes) with a slow degradation of the formed oxons consistent with the non-chlorinated controls. . The oxidative effect of 2 mg/L chlorine on the insecticides was essentially complete within 15 minutes (Table 2 and Figures 4,5 7,9,11). Chlorpyrifos and malathion were degraded 47%, azinphos methyl 49% and diazinon 53%. At 2 mg/L for 24 hours, chlorine degraded the four parent OP insecticides by 47 to 53%.

The effect of chlorine on the degradation of the diazinon oxon, azinphos methyl oxon, and malathion oxon was not apparent (Table 3 and Figures 4,6,8,10,12). Degradation of the insecticide oxons occurred at statistically the same levels as the control samples ($p=0.05$) (Figure 13). Approximately 9% of the chlorpyrifos oxon was degraded after 24 hours. These compounds appear less stable in dechlorinated, finished drinking water than the parents during 24-hour storage at ambient temperature. However, they appear to be resistant to chlorine degradation at 2 mg/L chlorine.

This observation was also seen by the degradation of the oxons that were formed during the parent insecticide degradation experiment (Figure 3). Primary oxon formation occurred within 30 minutes of exposure to 2 mg/L chlorine. Of the oxons formed, diazinon oxon was the greatest at 30% of the parent concentration at 30 minutes. This was followed by chlorpyrifos oxon (20% of parent), malathion oxon (15%) and azinphos methyl oxon (10%), (Table 2 and Figures 3,5,7,9,11). At 24 hours, diazinon oxon had degraded 21% from its peak concentration at 30 minutes, chlorpyrifos oxon degraded by 28%, malathion oxon 40% and azinphos methyl oxon 32%. The degradation percentages are essentially the same as those of oxon degradation in the 24 hour control samples (Figure 2).

Evaluation of Compound Degradation at 4 mg/L Chlorine Concentration

Parent insecticide degradation (and resultant oxon formation) and oxon degradation at each time interval is shown in Tables 4 and 5, and Figures 14 through 23.

Complete degradation (100%) of diazinon, malathion,. and azinphos methyl occurred within 15 minutes (Table 4 and Figures 14,16,18,20,22). Chlorpyrifos was 94% degraded within 15 minutes and the remaining was degraded within 30 minutes (Figure 18).

Oxon formation was evident with over 90% of the peak concentration of each oxon produced within 15 minutes for each analyte (Table 5 and Figures 14,16,18,20,22). Peak concentrations of each oxon were at the 30-minute time interval. Diazinon oxon concentration peaked at 60% of the parent concentration, chlorpyrifos oxon 74%,

malathion oxon 64% and azinphos methyl oxon 31%. Each oxon decreased by 21 to 31% over the remaining time period up to 24 hours. Azinphos methyl oxon formation was substantially less than the other degradates (Figure 14).

The degree of oxon degradation is not clearly defined at the 4 mg/L chlorine dosage, particularly with the malathion oxon and azinphos methyl oxon due to the loss of the oxons in the control samples (Figure 24). Statistically, the reduction of diazinon oxon and chlorpyrifos oxon was greater in the experimental samples than the controls, suggesting oxidative effects. Diazinon oxon in the control samples was reduced by 10% and in 4 mg/L chlorine samples 32%, chlorpyrifos oxon 15% and 40%, malathion oxon 45% and 50%. No significant difference was noted for malathion oxon (45% and 50%) and azinphos methyl oxon 38% and 39%. In finished drinking water at 4 mg/L chlorine, diazinon and chlorpyrifos oxons slightly degrade through chlorine oxidation. Malathion and azinphos methyl oxons are not affected by chlorine oxidation. The oxons are much more stable than the parent insecticides in the presence of 4 mg/L chlorine in finished drinking water. Chemical processes other than chlorine oxidation are causing degradation of the malathion and azinphos methyl oxons.

Phase 2: Evaluation of acephate and methamidophos

Evaluation of Compound Stability in Control Samples

The stability of the target analytes was evaluated in the control samples over the 24-hour time interval using a two-sample *t* test for independent variables with equal variances (two-sided) at the 95% confidence interval (figure 25). Acephate degraded by approximately 7% and methamidophos degraded by approximately 14%. Methamidophos was not detected in the 24-hour, acephate spike control.

Evaluation of Compound Degradation at 2 mg/L Chlorine Concentration

Partial degradation of acephate and methamidophos was noted over 24 hours (Table 6 and Figures 26,27,28,29). Acephate degraded to 57% and methamidophos 59% of their original concentrations over 24 hours in 2 mg/L chlorine finished drinking water. The degradation effect of chlorine on the compounds was complete within 60 minutes. Approximately 35% of the acephate and 31% of the methamidophos was degraded after 24 hours as compared to the 24-hour control sample.

Evaluation of Compound Degradation at 4 mg/L Chlorine Concentration

Degradation of acephate occurred within 15 minutes (Table 7 and Figures 30 and 32). Methamidophos was not created from the degradation of the acephate, or methamidophos was completely degraded by the chlorine upon formation (Table 7 and Figures 31 and 33). Methamidophos degradation was less rapid, with 67% removal within 15 minutes (Figure 31). After 60 minutes 5% of the methamidophos remained, degradation was complete after 24 hours of contact.

Because of its persistence beyond 15 minutes in 4 mg/L chlorine water, methamidophos appears more resistant to chlorine degradation than acephate. The stability of methamidophos at a 4 mg/L chlorine concentration further indicates that methamidophos was not formed resulting from acephate degradation. This stability should have resulted in an observation of methamidophos prior to it degrading if it was formed from acephate degradation.

Impact of Chlorine and Chloramines

The partial degradation (47 to 53%) of chlorpyrifos, diazinon, malathion, and azinphos methyl at a 2 mg/L chlorine concentration was surprising in lieu of the relatively high concentration of chlorine as compared to the initial target analyte concentrations. This is especially surprising considering the rapid degradation of a large percentage of the parent compounds after 15 minutes of contact followed by little or no continued degradation throughout the remaining 24 hours of contact time.

During the experimental phase investigating the chlorine degradation of acephate and methamidophos, total and free chlorine measurements were performed to determine the ratio of free and combined (chloramines) chlorine in the potable water. It was determined that at a 2 mg/L chlorine concentration, the total chlorine existed as combined chlorine. At a 4 mg/L chlorine concentration, the combined and free chlorine concentrations were approximately 2 mg/L each.

The total chlorine in the potable water from the chlorination process at the treatment plant was quenched using sodium thiosulfate. The chlorine (combined and free) was completely removed by the thiosulfate. Addition of sodium hypochlorite to the potable water resulted in the reformation of chloramines by reaction with the ammonium ion present in the tap water. This occurred at both 2 and 4 mg/L chlorine concentrations. However, at 4 mg/L not all chlorine was present as chloramines, half the concentration (2mg/L) was present as free chlorine.

From a disinfection standpoint, chloramines exert less oxidative power than free chlorine (hypochlorite). Correspondingly, chloramines may have a less degradative effect on the target insecticides and this may have contributed to the lower degradative effect seen at the 2 mg/L chlorine concentration. This study evaluated the effect of total free and combined chlorine. Future studies are recommended to evaluate the oxidative effect of free and combined chlorines on the target insecticides and their oxons.

CONCLUSIONS

Chlorine (sodium hypochlorite) at an initial concentration of 2 mg/L exerts an oxidative effect on the parent insecticides. These effects range from 47 to 53% decrease in parent insecticide present. The oxidative effects are essentially complete within 15 minutes with the exception of acephate (60 minutes) and methamidophos. Continued methamidophos degradation during the 24 hour experiment was evident.

The percentage of the parent insecticides degrading after 24 hours of contact time was as follows: diazinon 53%, chlorpyrifos 47%, malathion 47%, azinphos methyl 49%, acephate 43% and methamidophos 41%. Insecticide oxon formation occurred during parent degradation. As a percent of parent concentration diazinon oxon was present at 30 percent, chlorpyrifos oxon at 20%, malathion oxon at 15% and azinphos methyl at 10%.

Chlorine degradation of diazinon oxon, malathion oxon and azinphos methyl oxon did not occur at a chlorine concentration 2mg/L. Chlorpyrifos oxon was degraded by 9% as compared to the control sample.

At a chlorine concentration of 4 mg/L there was no degradation of malathion oxon or azinphos methyl oxon. Diazinon oxon and chlorpyrifos oxon were degraded 22% and 25% respectively in 4 mg/L chlorine water over 24 hours.

Chlorine (sodium hypochlorite) at an initial concentration of 4 mg/L completely degrades diazinon, malathion, azinphos methyl, and acephate within 15 minutes of contact time. Chlorpyrifos is 94% degraded within 15 minutes and 100% within 30 minutes. Methamidophos degradation was 69% complete after 15 minutes, 95% after 60 minutes and 100% by 24 hours of contact.

All oxons are significantly more resistant to chlorine degradation compared to the parent compounds. However, the data indicates that the oxons are more susceptible to reduction during 24-hour storage at ambient temperature than the parent compounds, particularly for malathion oxon and azinphos methyl oxon. A process other than oxidation is causing partial degradation of the insecticide oxons.

TABLE 1

METHOD DETECTION LIMIT FOR SAMPLES ANALYZED BY GC/MS-SIM AND GC/FPD

Compound	No. of Reps	Spike Level, ug/l	Average Recovery, ug/l (% R)	Deviation, ug/l (%CV)	MDL, ug/l	PQL, ug/l
Extracts Analyzed by GC/MS-SIM						
C-18 Extraction						
Diazinon Oxon	15	0.050	0.0461 (92%)	0.0031 (6.7%)	0.0088	0.050
Diazinon	15	0.050	0.0576 (115%)	0.0025 (4.4%)	0.0058	0.050
Malathion Oxon	15	0.050	0.0432 (86%)	0.0028 (6.5%)	0.0085	0.050
Malathion	15	0.050	0.0488 (98%)	0.0032 (6.5%)	0.0086	0.050
Chlorpyrifos Oxon	15	0.050	0.0552 (110%)	0.0029 (5.3%)	0.0070	0.050
Chlorpyrifos	15	0.050	0.0388 (78)%	0.0026 (6.8%)	0.0089	0.050
Azinphos-methyl Oxon	15	0.050	0.0374 (75%)	0.0037 (10.0%)	0.0131	0.065
Azinphos-methyl	15	0.050	0.0336 (67%)	0.0026 (7.7%)	0.0100	0.050
Extracts Analyzed by GC/FPD						
Methamidophos	8	0.030	0.0195 (65%)	0.0011 (5.6%)	0.0052	0.050
Acephate	8	0.030	0.0210 (70%)	0.0018 (8.6%)	0.0079	0.050

TABLE 2
PARENT INSECTICIDES AND THEIR DEGRADATES AT 2 mg/L CHLORINE IN FINISHED DRINKING WATER (ug/l)

	T = 0 min					T = 15 min					T = 30 min				
Parent Spikes	R1	R2	R3	Ave.	Std. Dev.	R1	R2	R3	Ave.	Std. Dev.	R1	R2	R3	Ave.	Std. Dev.
Diazinon	394	370	398	387	15	184	180	182	182	2	152	180	180	171	16
Diazinon Oxon	nd	nd	nd	---	---	122	114	118	118	4	112	128	130	123	10
Chlorpyrifos	386	344	390	373	25	248	234	272	251	19	212	236	230	226	12
Chlorpyrifos Oxon	nd	nd	nd	---	---	76	72	74	74	2	70	80	84	78	7
Malathion	432	390	434	419	25	250	232	234	239	10	206	234	228	223	15
Malathion Oxon	nd	nd	nd	---	---	62	64	64	63	1	66	76	76	73	6
Azinphos Methyl	414	386	412	404	16	244	198	210	217	24	182	212	214	203	18
Azinphos Methyl Oxon	nd	nd	nd	---	---	50	34	36	40	9	38	42	44	41	3

	T = 60 min					T = 24 hr					T = 24 hr Control				
Parent Spikes	R1	R2	R3	Ave.	Std. Dev.	R1	R2	R3	Ave.	Std. Dev.	R1	R2	R3	Ave.	Std. Dev.
Diazinon	190	200	192	194	5	186	174	190	183	8	376	370	382	376	6
Diazinon Oxon	106	106	98	103	5	100	94	98	97	3	nd	nd	nd	—	—
Chlorpyrifos	242	246	248	245	3	204	184	206	198	12	370	342	366	359	15
Chlorpyrifos Oxon	66	62	68	65	3	56	54	58	56	2	nd	nd	nd	—	—
Malathion	244	248	242	245	3	226	210	224	220	9	332	290	328	317	23
Malathion Oxon	50	56	46	51	5	46	44	40	43	3	nd	nd	nd	—	—
Azinphos Methyl	216	204	216	212	7	204	204	206	205	1	388	318	390	365	41
Azinphos Methyl Oxon	26	28	26	27	1	28	26	30	28	2	nd	nd	nd	—	—

TABLE 3
INSECTICIDE OXONS AT 2 mg/L CHLORINE IN FINISHED DRINKING WATER (ug/l)

	T = 0 min					T = 15 min					T = 30 min				
Oxon Spikes	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**
Diazinon Oxon	420	408	376	**401**	**23**	400	396	406	**401**	**5**	392	374	388	385	9
Chlorpyrifos Oxon	394	400	396	**397**	**3**	356	340	352	**349**	**8**	374	356	388	373	16
Malathion Oxon	408	382	388	**393**	**14**	318	304	318	**313**	**8**	336	330	356	341	14
Azinphos Methyl Oxon	398	380	394	**391**	**9**	294	274	298	**289**	**13**	322	328	338	329	8

	T = 60 min					T = 24 hr					T = 24 hr Control				
Oxon Spikes	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**
Diazinon Oxon	374	394	384	**384**	**10**	392	358	344	**365**	**25**	344	364	370	359	14
Chlorpyrifos Oxon	338	342	338	**339**	**2**	314	300	306	**307**	**7**	326	334	350	337	12
Malathion Oxon	302	306	286	**298**	**11**	246	226	226	**233**	**12**	198	220	234	217	18
Guthion Oxon	300	304	318	**307**	**9**	244	274	278	**265**	**19**	180	264	284	243	55

TABLE 4
PARENT INSECTICIDES AND THEIR DEGRADATES AT 4 mg/L CHLORINE IN FINISHED DRINKING WATER (ug/l)

	T = 0 min					T = 15 min					T = 30 min				
Parent Spikes	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**
Diazinon	394	370	398	**387**	**15**	nd	nd	nd	—	—	nd	nd	nd	—	—
Diazinon Oxon	nd	nd	nd	---	---	232	214	222	**223**	**9**	224	236	232	231	6
Chlorpyrifos	386	344	390	**373**	**25**	22	22	22	**22**	**0**	nd	nd	nd	—	—
Chlorpyrifos Oxon	nd	nd	nd	---	---	262	244	248	**251**	**9**	268	290	268	275	13
Malathion	432	390	434	**419**	**25**	nd	nd	nd	—	—	nd	nd	nd	—	—
Malathion Oxon	nd	nd	nd	---	---	240	250	254	**248**	**7**	266	278	262	269	8
Azinphos Methyl	414	386	412	**404**	**16**	nd	nd	nd	—	—	nd	nd	nd	—	—
Azinphos Methyl Oxon	nd	nd	nd	---	---	68	104	108	**93**	**22**	126	134	120	127	7

	T = 60 min					T = 24 hr					T = 24 hr Control				
Parent Spikes	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**
Diazinon	nd	nd	nd	—	—	nd	nd	nd	—	—	376	370	382	376	6
Diazinon Oxon	200	218	224	214	**12**	184	186	180	**183**	**3**	nd	nd	nd	—	—
Chlorpyrifos	nd	nd	nd	—	—	nd	nd	nd	—	—	370	342	366	359	15
Chlorpyrifos Oxon	240	262	268	257	**15**	200	212	204	**205**	**6**	nd	nd	nd	—	—
Malathion	nd	nd	nd	—	—	nd	nd	nd	—	—	332	290	328	317	23
Malathion Oxon	216	250	262	243	**24**	182	188	180	**183**	**4**	nd	nd	nd	—	—
Azinphos Methyl	nd	nd	nd	—	—	nd	nd	nd	—	—	388	318	390	365	41
Azinphos Methyl Oxon	88	100	126	105	**19**	82	88	92	**87**	**5**	nd	nd	nd	—	—

TABLE 5
INSECTICIDE OXONS AT 4 mg/L CHLORINE IN FINISHED DRINKING WATER (ug/l)

	T = 0 min					T = 15 min					T = 30 min				
Oxon Spikes	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**
Diazinon Oxon	420	408	376	**401**	**23**	358	358	342	**353**	**9**	322	296	306	308	13
Chlorpyrifos Oxon	394	400	396	**397**	**3**	332	342	338	**337**	**5**	294	282	294	290	7
Malathion Oxon	408	382	388	**393**	**14**	274	294	292	**287**	**11**	268	258	282	269	12
Azinphos Methyl Oxon	398	380	394	**391**	**9**	198	212	266	**225**	**36**	298	286	308	297	11

	T = 60 min					T = 24 hr					T = 24 hr Control				
Oxon Spikes	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**	**R1**	**R2**	**R3**	**Ave.**	**Std. Dev.**
Diazinon Oxon	332	300	296	**309**	**20**	284	266	268	**273**	**10**	344	364	370	359	14
Chlorpyrifos Oxon	314	290	284	**296**	**16**	254	228	232	**238**	**14**	326	334	350	337	12
Malathion Oxon	284	254	250	**263**	**19**	206	192	196	**198**	**7**	198	220	234	217	18
Guthion Oxon	316	258	260	**278**	**33**	250	224	240	**238**	**13**	180	264	284	243	55

TABLE 6
ACEPHATE AND METHAMIDOPHOS AT 2 mg/l CHLORINE IN FINISHED DRINKING WATER (ug/l)

	T = 0 min					T = 15 min					T = 30 min				
Acephate Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Acephate	462	443	396	434	8%	369	359	335	354	5%	343	400	414	386	10%
Methamidophos	nd	nd	nd	---	---	nd	nd	nd	---	---	nd	nd	nd	---	---

	T = 60 min					T = 24 hr					T = 24 hr Control				
Acephate Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Acephate	208	198	337	248	31%	358	214	213	262	32%	464	424	319	402	19%
Methamidophos	nd	nd	nd	---	---	nd	nd	nd	---	---	nd	nd	nd	---	---

	T = 0 min					T = 15 min					T = 30 min				
Methamidphos Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Methamidophos	413	432	302	382	18%	325	338	363	342	6%	287	276	338	300	11%

	T = 60 min					T = 24 hr					T = 24 hr Control				
Methamidphos Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Methamidophos	306	168	302	259	30%	247	321	106	225	49%	382	281	319	327	16%

TABLE 7
ACEPHATE AND METHAMIDOPHOS AT 4 mg/l CHLORINE IN FINISHED DRINKING WATER (ug/l)

	T = 0 min					T = 15 min					T = 30 min				
Acephate Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Acephate	462	443	396	434	8%	nd	nd	nd	---	---	nd	nd	nd	---	---
Methamidophos	nd	nd	nd	---	---	nd	nd	nd	---	---	nd	nd	nd	---	---

	T = 60 min					T = 24 hr					T = 24 hr Control				
Acephate Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Acephate	nd	nd	nd	---	---	nd	nd	nd	---	---	464	424	319	402	19%
Methamidophos	nd	nd	nd	---	---	nd	nd	nd	---	---	nd	nd	nd	---	---

	T = 0 min					T = 15 min					T = 30 min				
Methamidphos Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Methamidophos	413	432	302	382	18%	113	124	143	127	12%	85	74	84	81	8%

	T = 60 min					T = 24 hr					T = 24 hr Control				
Methamidphos Spike	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv	R1	R2	R3	Ave.	%cv
Methamidophos	25	17	19	20	20%	nd	nd	nd	---	---	382	281	319	327	16%

FIGURE 1
24HR CONTROL SAMPLES, PARENT INSECTICIDES DEGRADATION AND FORMATION OF OXON DEGRADATES (% REMAINING)

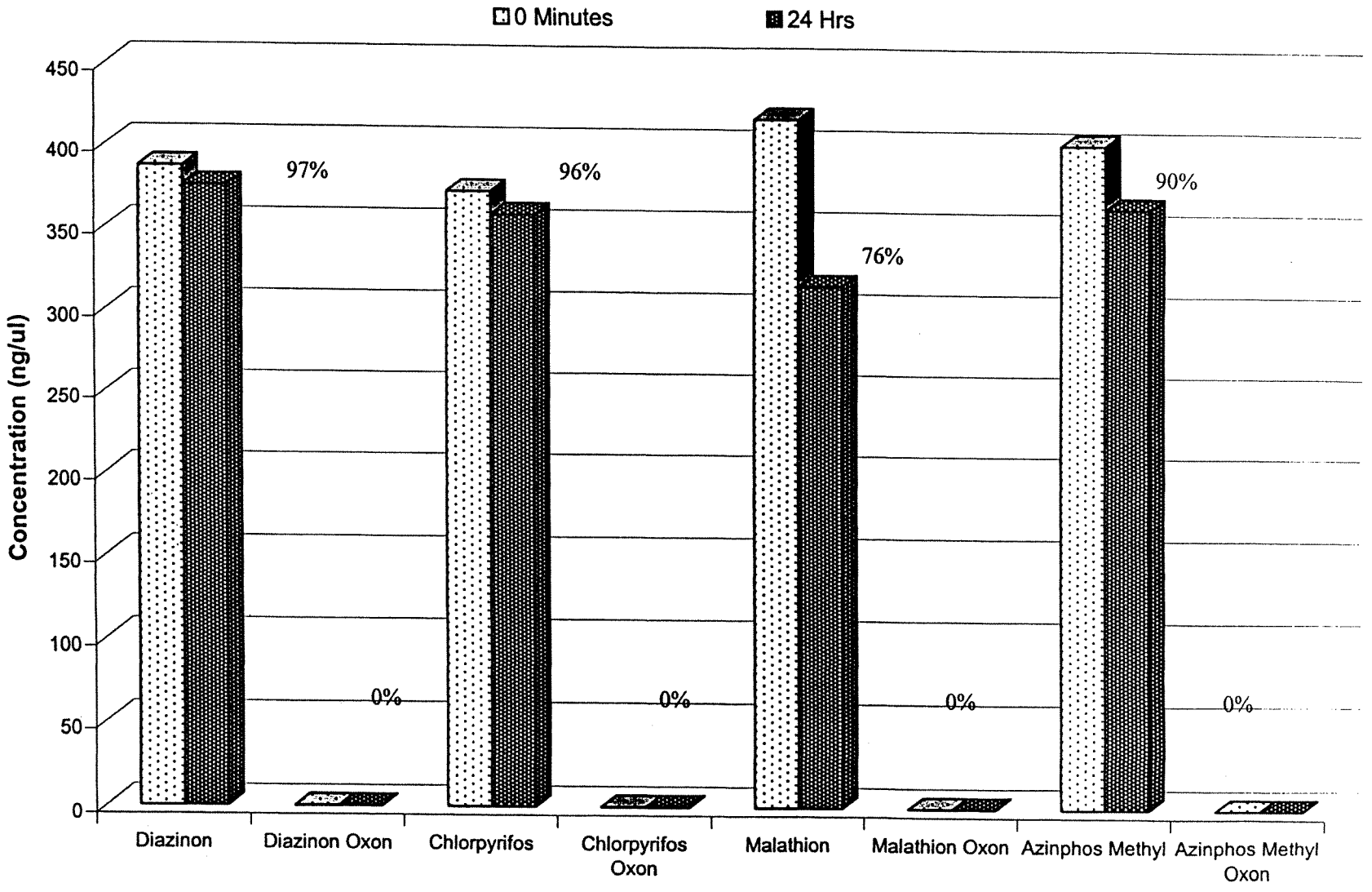


FIGURE 2
24 HR CONTROL SAMPLES, INSECTICIDE OXONS DEGRADATION OVER 24 HOURS
(% REMAINING)

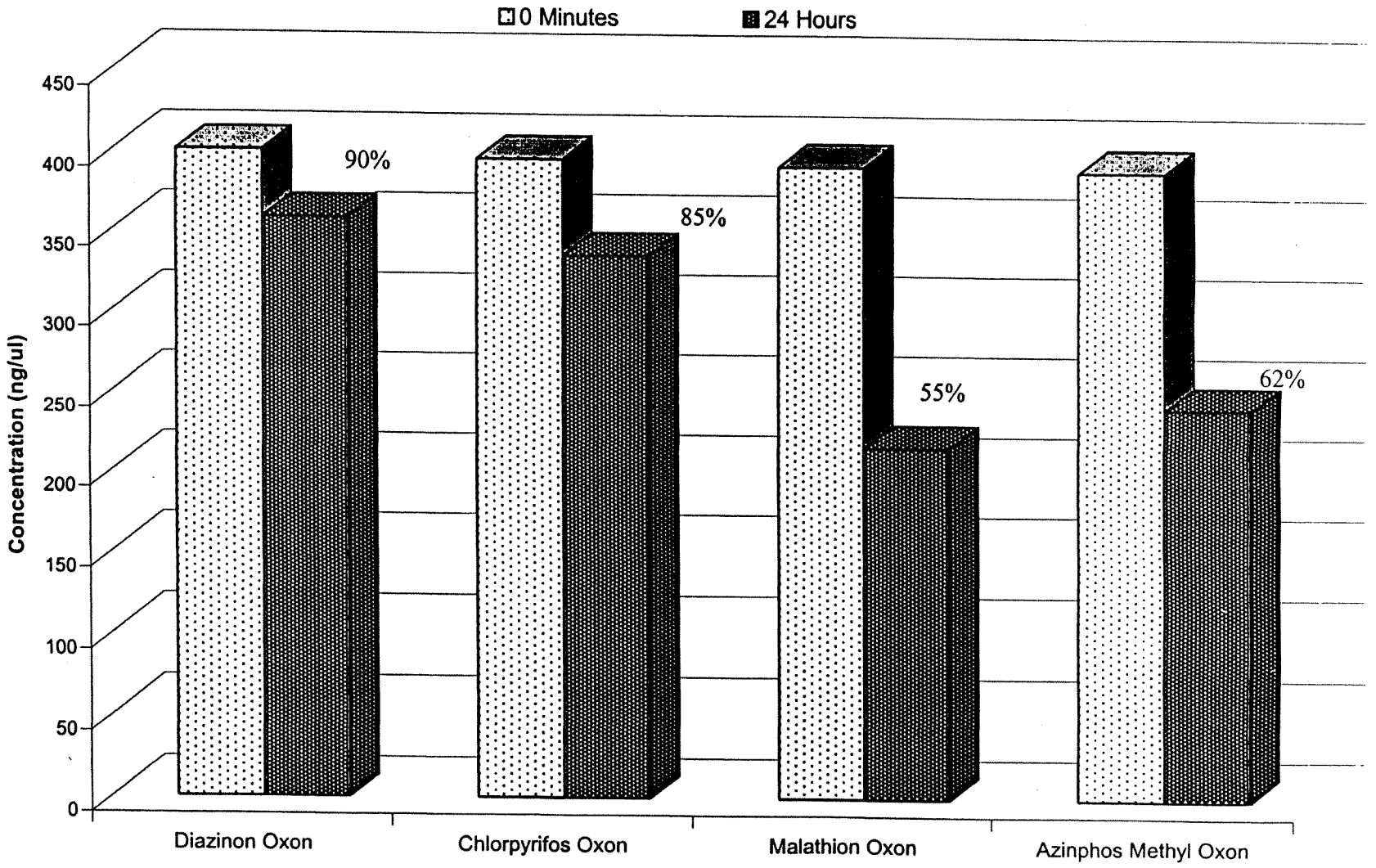


FIGURE 3
DEGRADATION OF PARENT INSECTICIDES AND FORMATION OF OXONS AT 2 mg/L CHLORINE CONCENTRATION (%REMAINING AT 24 HRS)

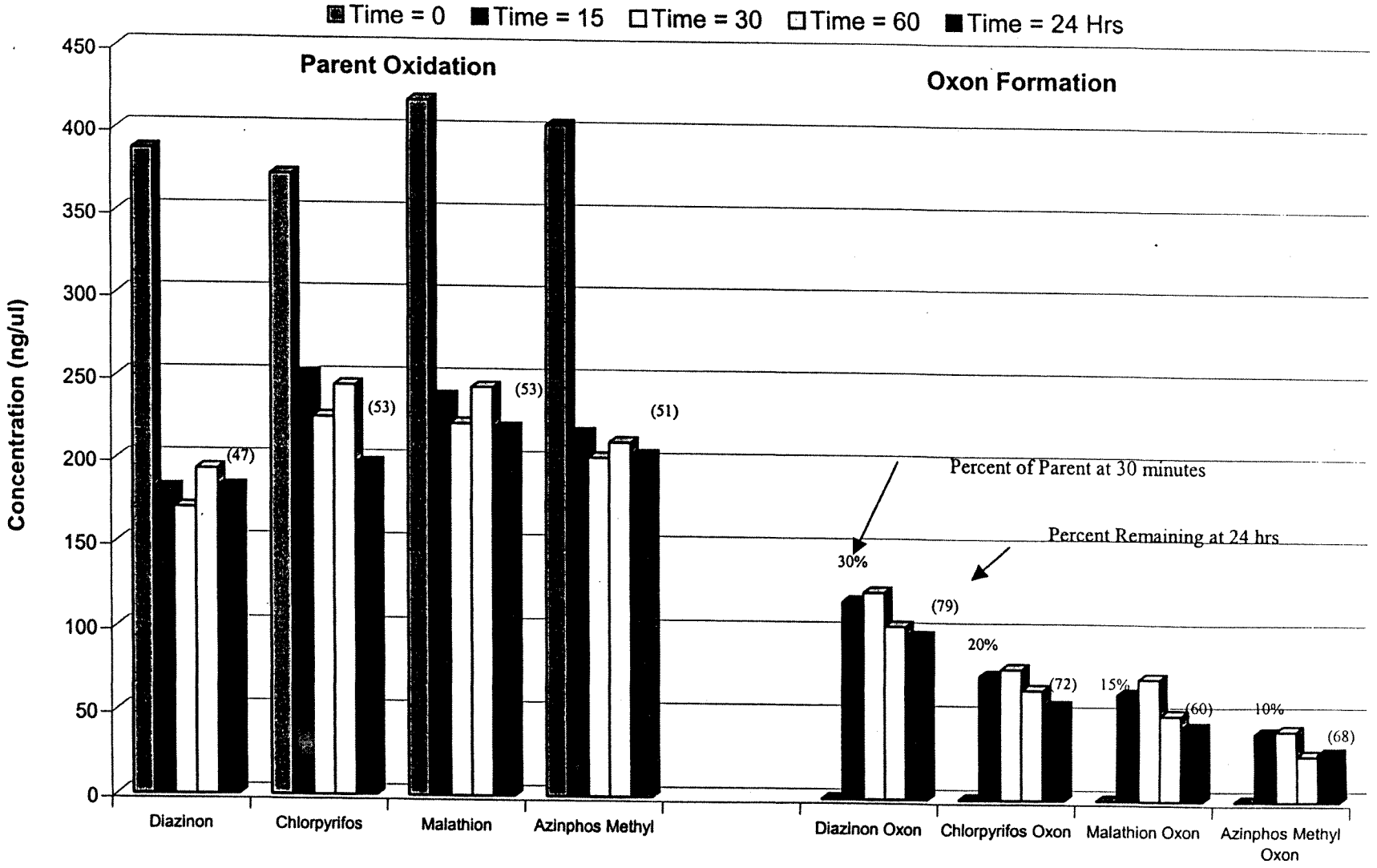


FIGURE 4
DEGRADATION OF INSECTICIDE OXONS AT 2 mg/L CHLORINE CONCENTRATION
(% OXON REMAINING)

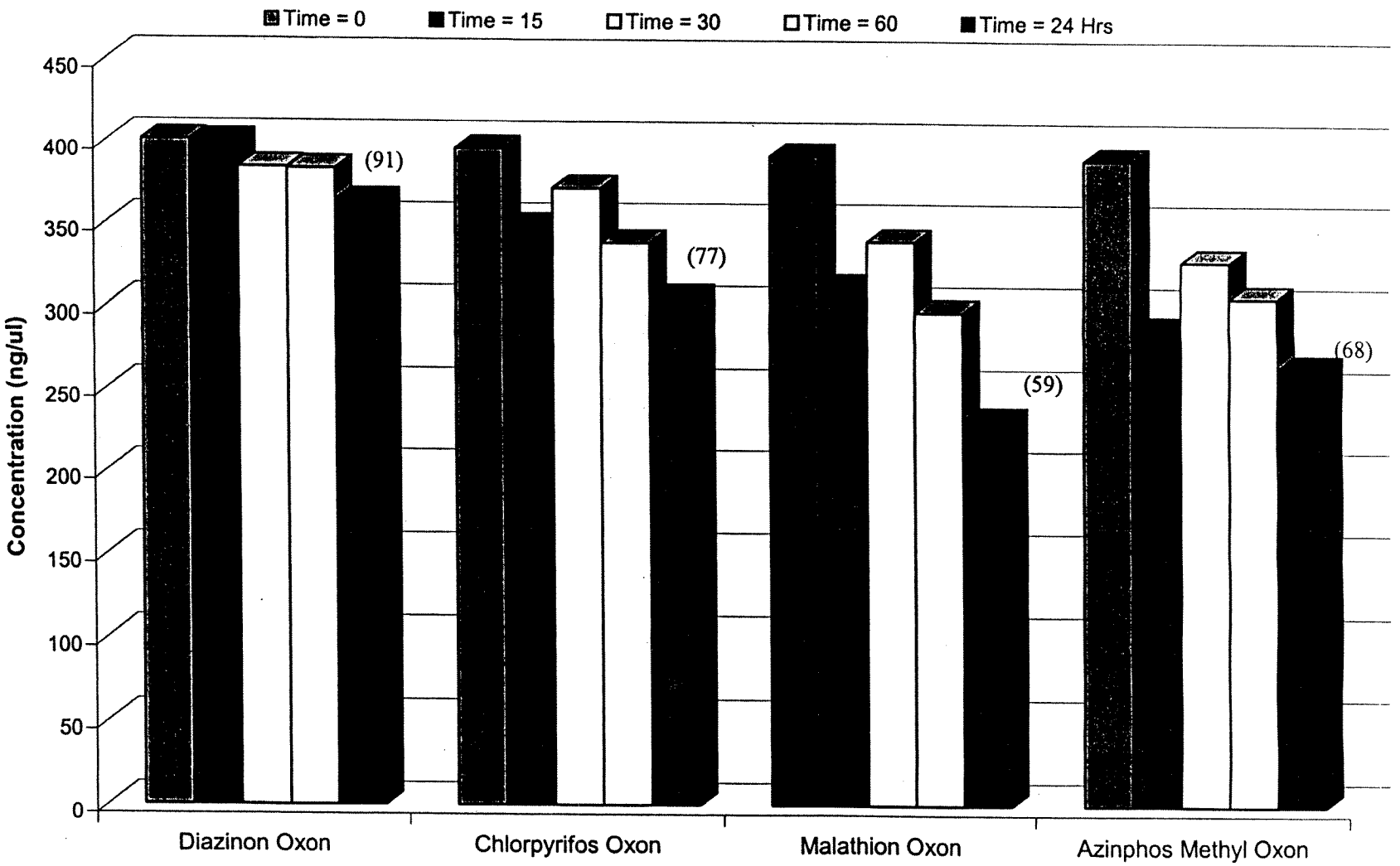


Figure 5
Diazinon Degradation and Oxon Formation at 2.0 mg/L
Chlorine in Finished Drinking Water

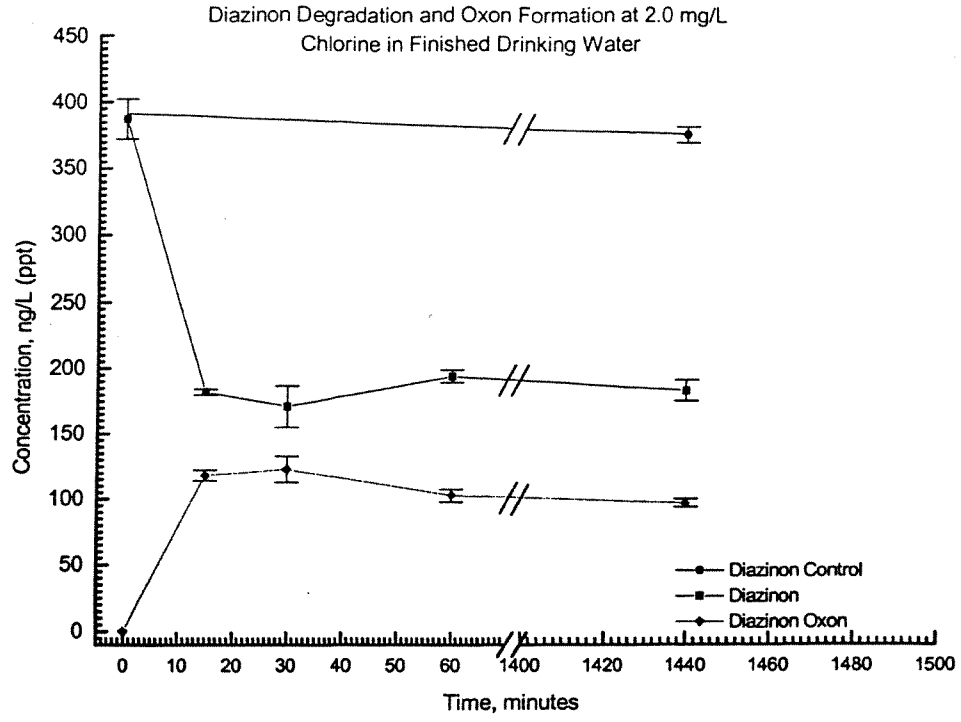


Figure 6
Diazinon Oxon Degradation at 2.0 mg/L
Chlorine in Finished Drinking Water

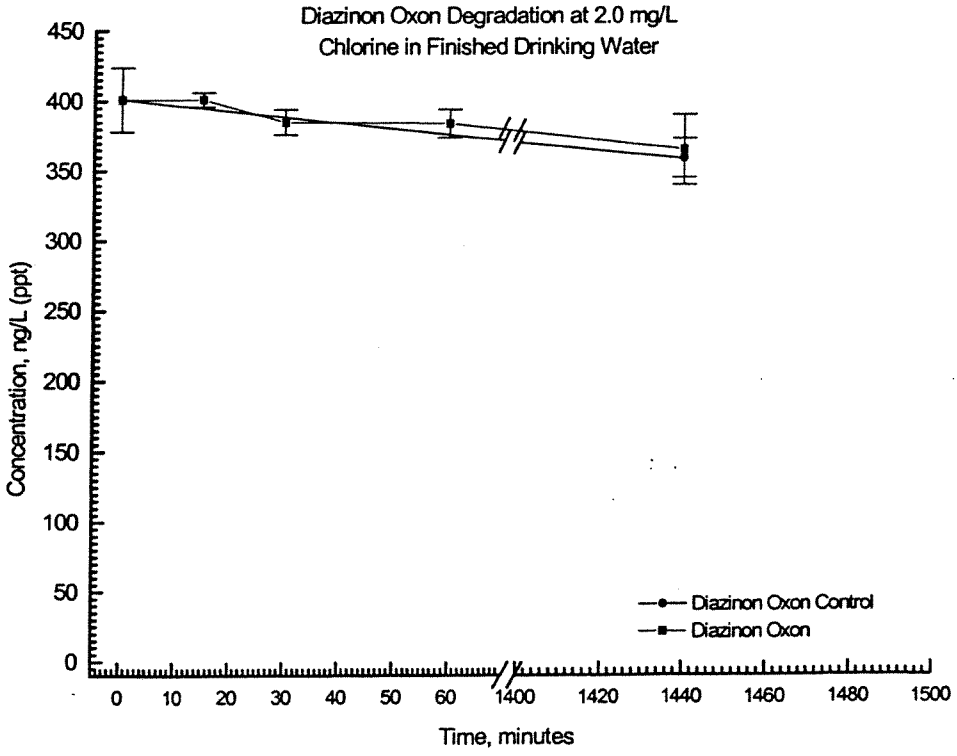


Figure 7
Chlorpyrifos Degradation and Oxon Formation
at 2.0 mg/L Chlorine in Finished Drinking Water

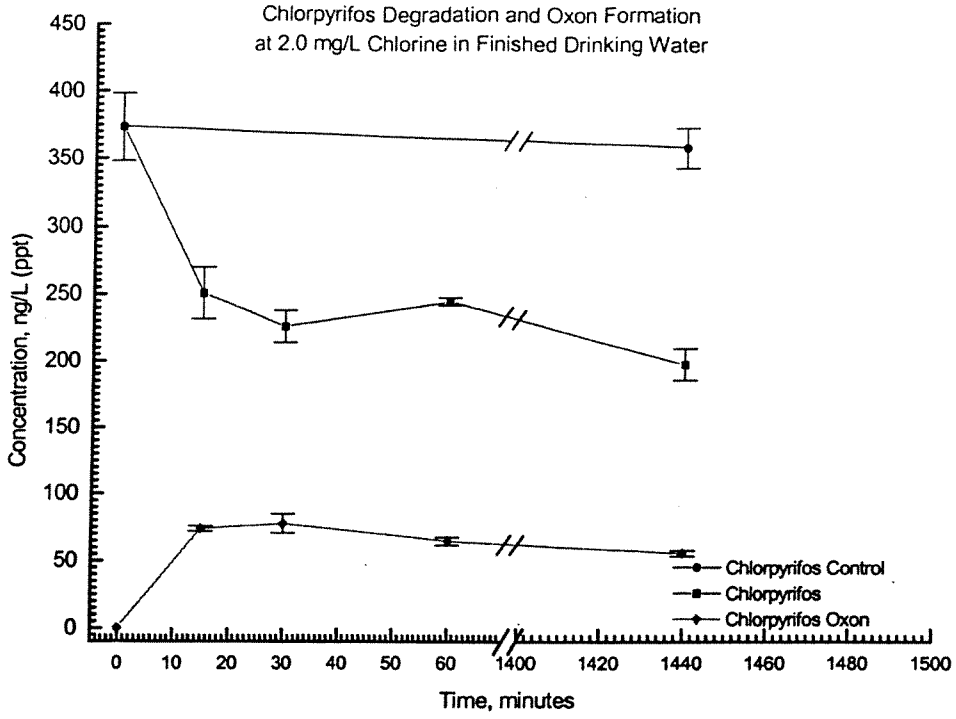


Figure 8
Chlorpyrifos Oxon Degradation at 2.0 mg/L
Chlorine in Finished Drinking Water

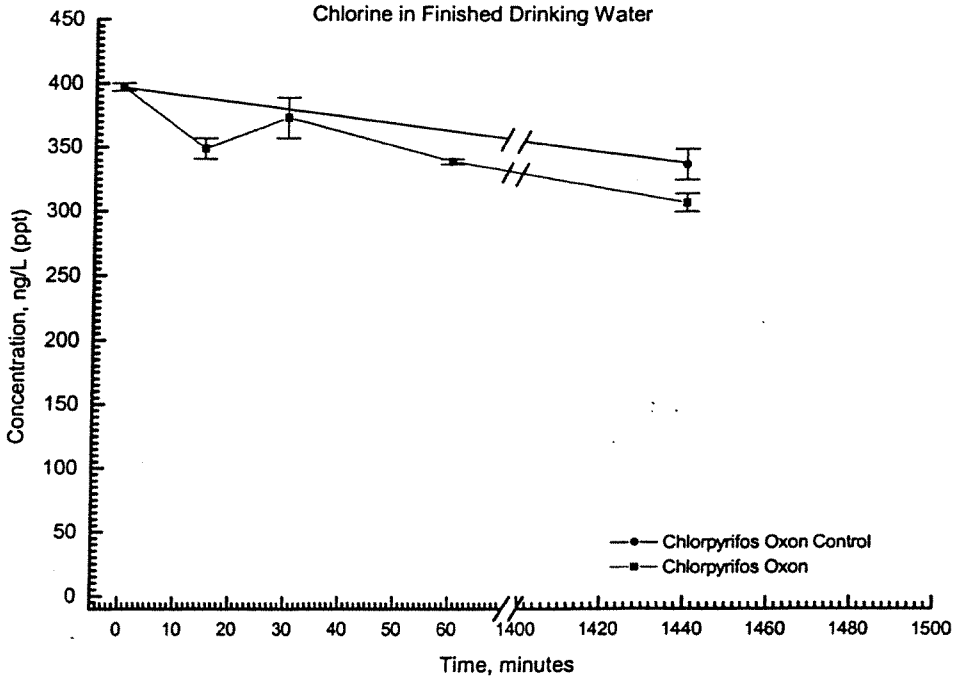


Figure 9
Malathion Degradation and Oxon Formation at
2.0 mg/L Chlorine in Finished Drinking Water

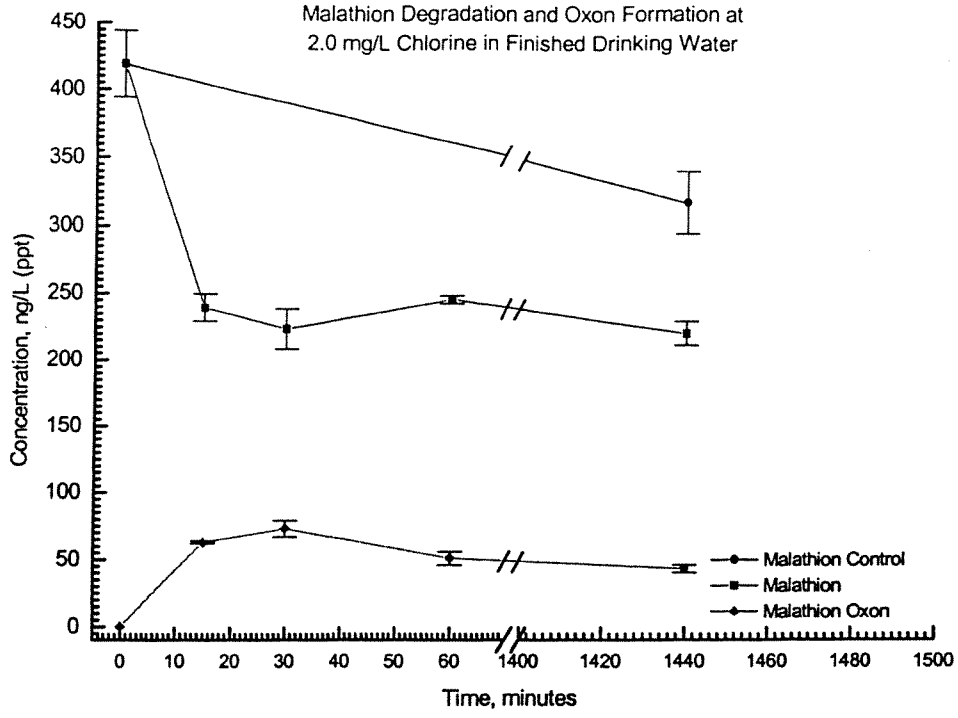


Figure 10
Malathion Oxon Degradation at 2.0 mg/L
Chlorine in Finished Drinking Water

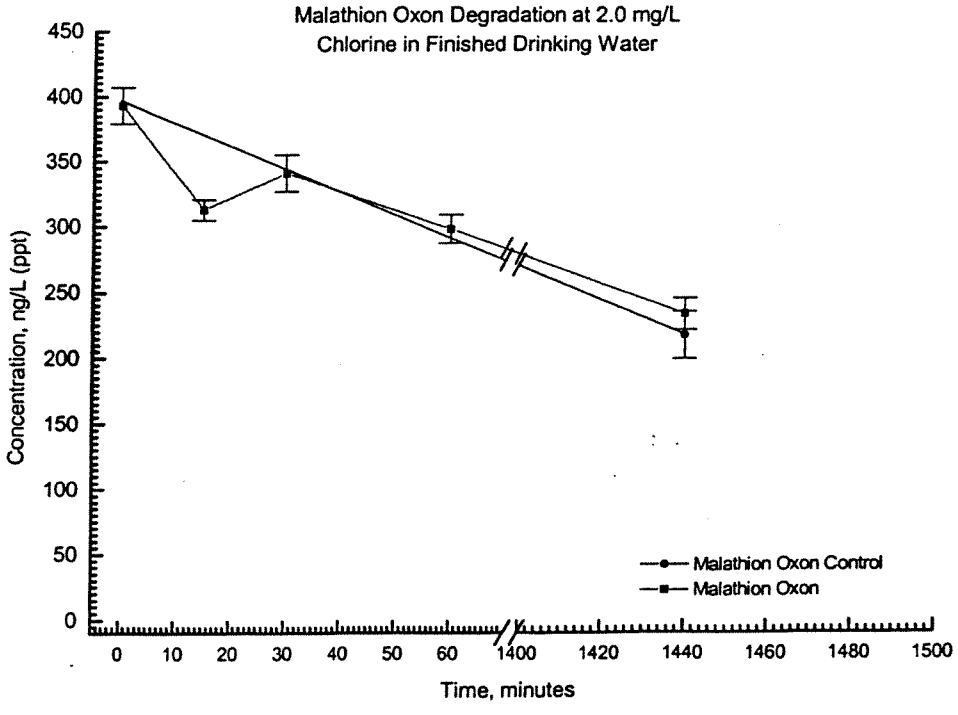


Figure 11
Azinphos Methyl Degradation and Oxon Formation at 2.0 mg/L
Chlorine in Finished Drinking Water

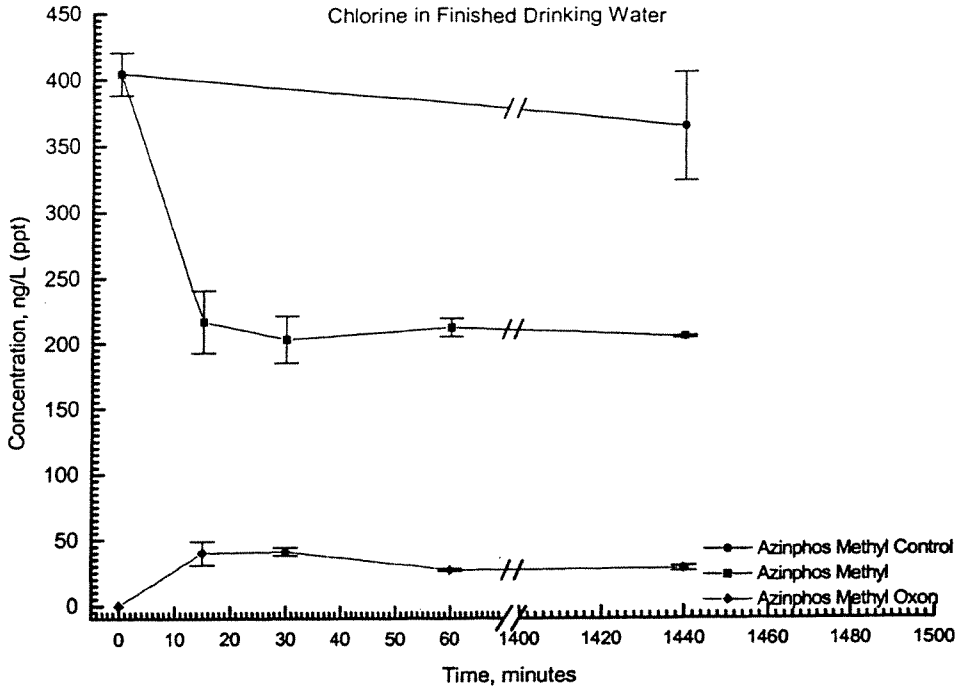


Figure 12
Azinphos Methyl Oxon Degradation and Oxon Formation at 2.0 mg/L
Chlorine in Finished Drinking Water

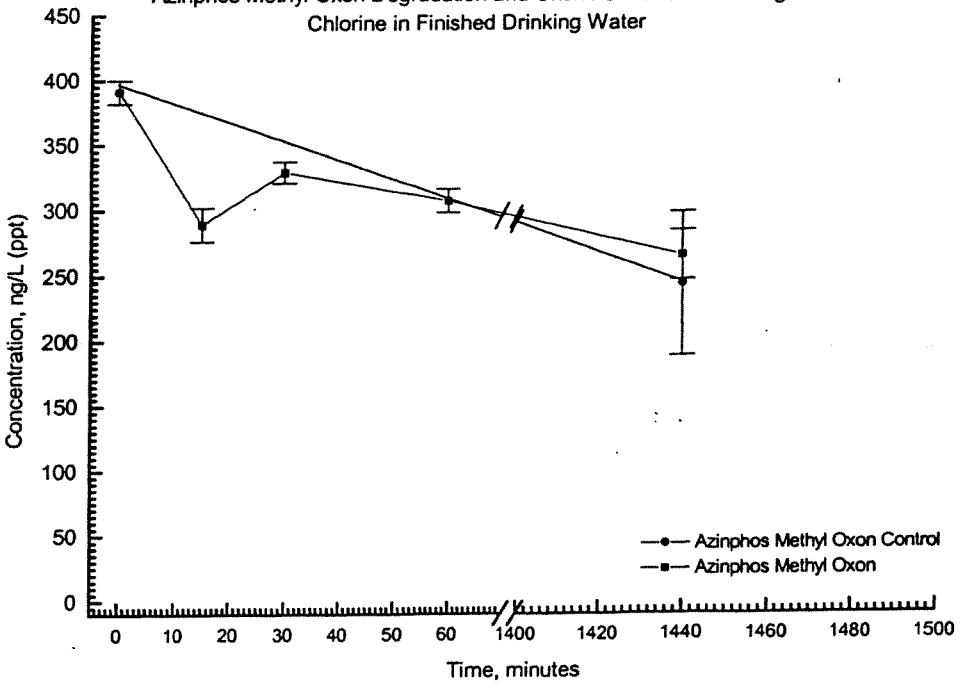


FIGURE 13
OXON DEGREDATION IN CONTROL VERSUS EXPERIMENTAL SAMPLES AFTER 24 HOURS, 2 mg/L CHLORINE CONCENTRATION

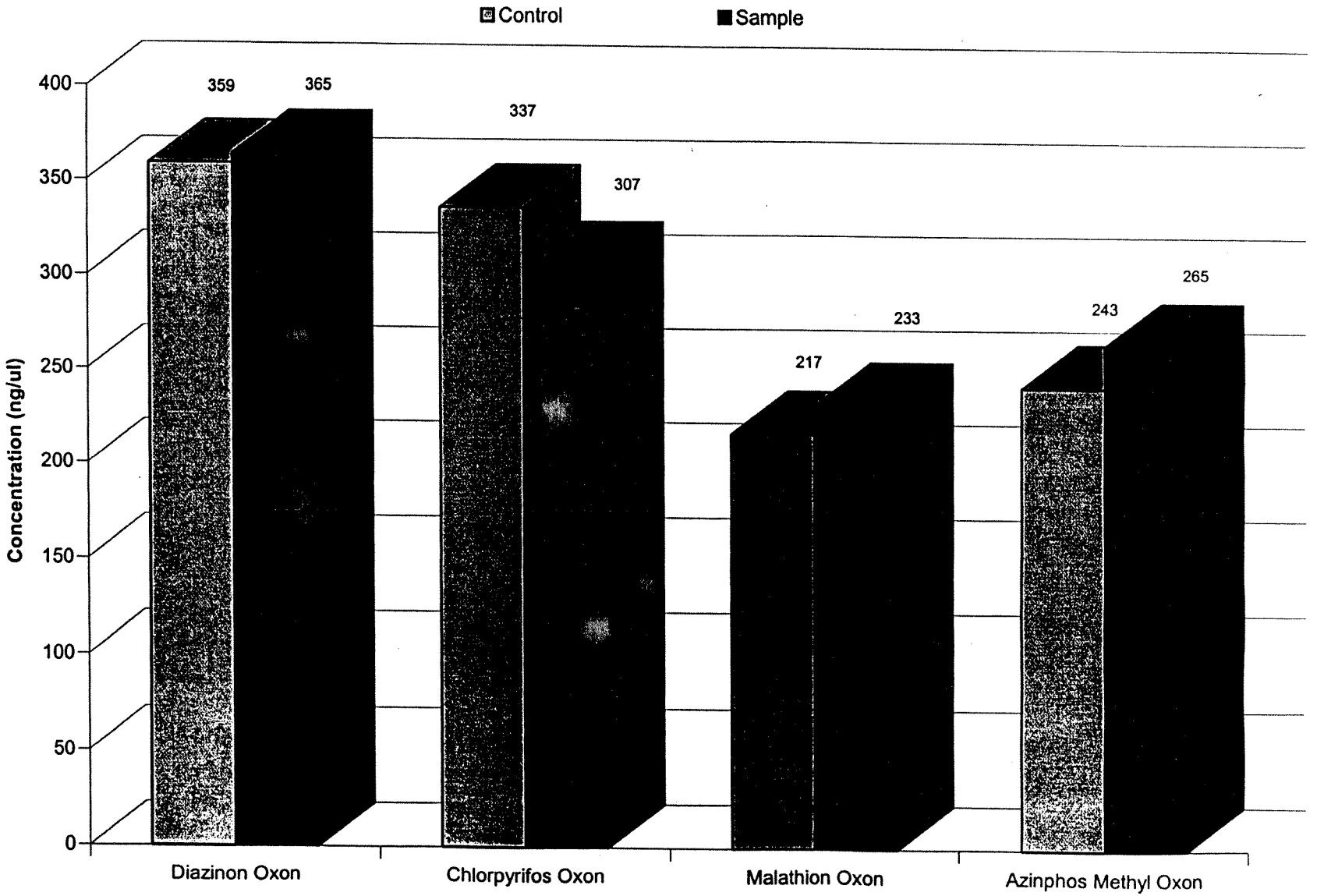


FIGURE 14
DEGRADATION OF PARENT INSECTICIDES AND FORMATION OF OXONS AT 4 mg/L CHLORINE CONCENTRATION

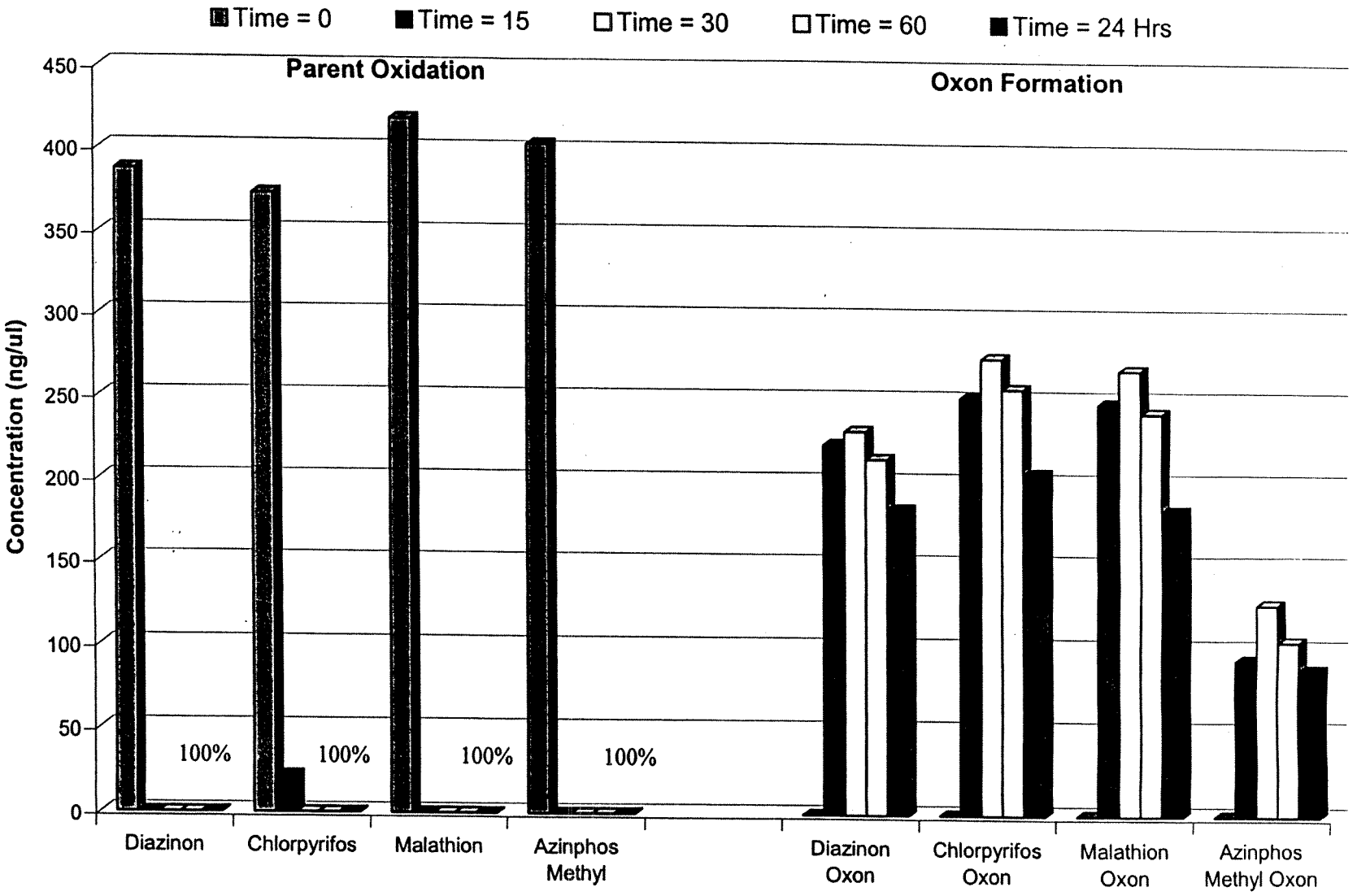


FIGURE 15
DEGRADATION OF INSECTICIDE OXONS AT 4 mg/L CHLORINE CONCENTRATION
(% REMAINING)

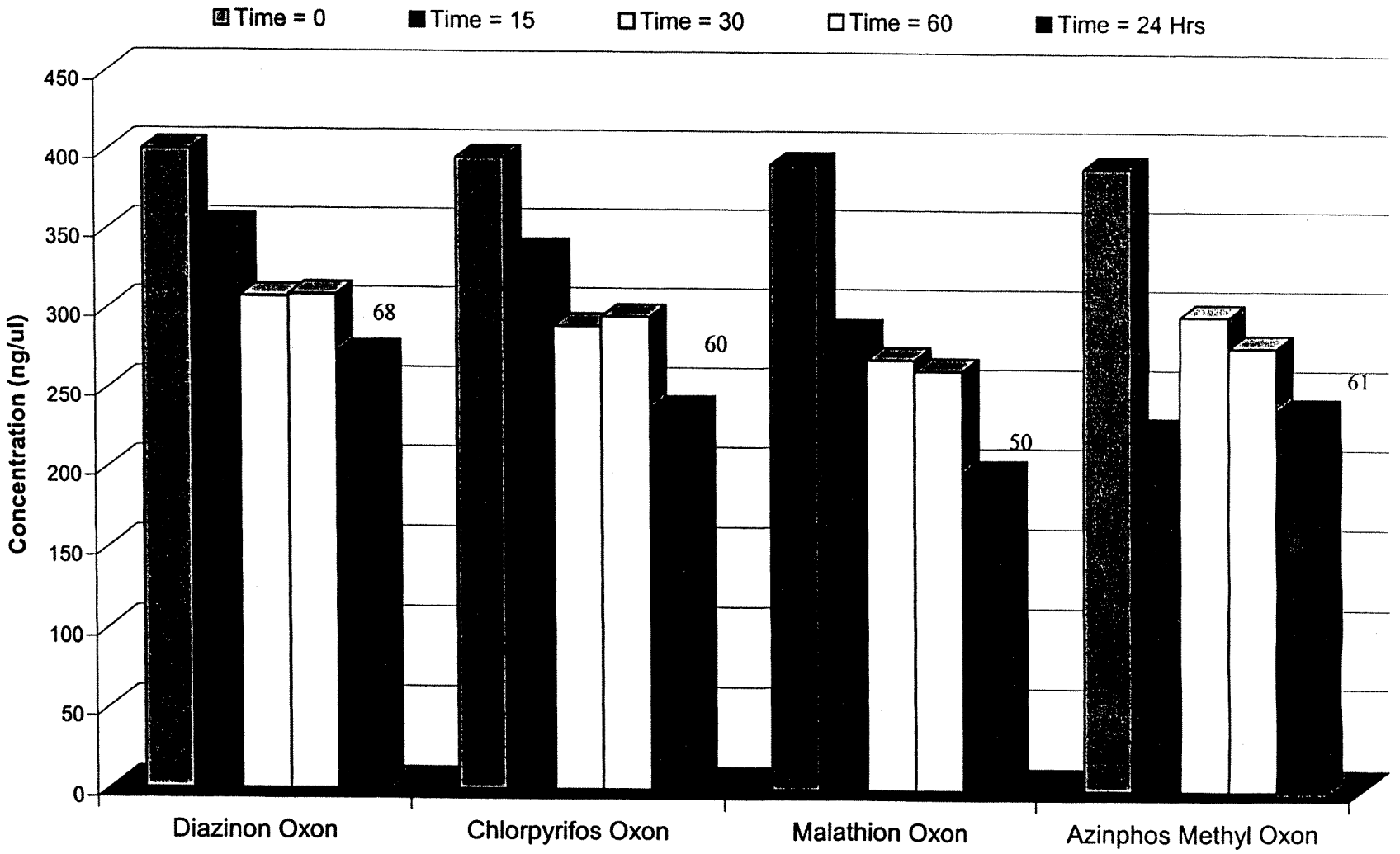


Figure 16
Diazinon Degradation and Oxon Formation at 4.0 mg/L
Chlorine in Finished Drinking Water

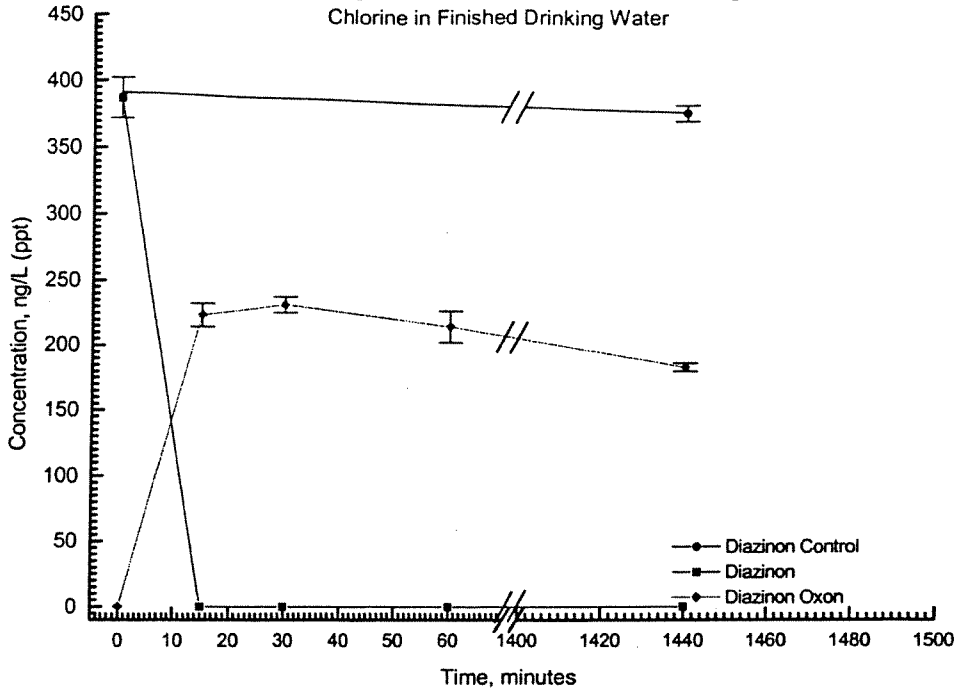


Figure 17
Diazinon Oxon Degradation at 4.0 mg/L
Chlorine in Finished Drinking Water

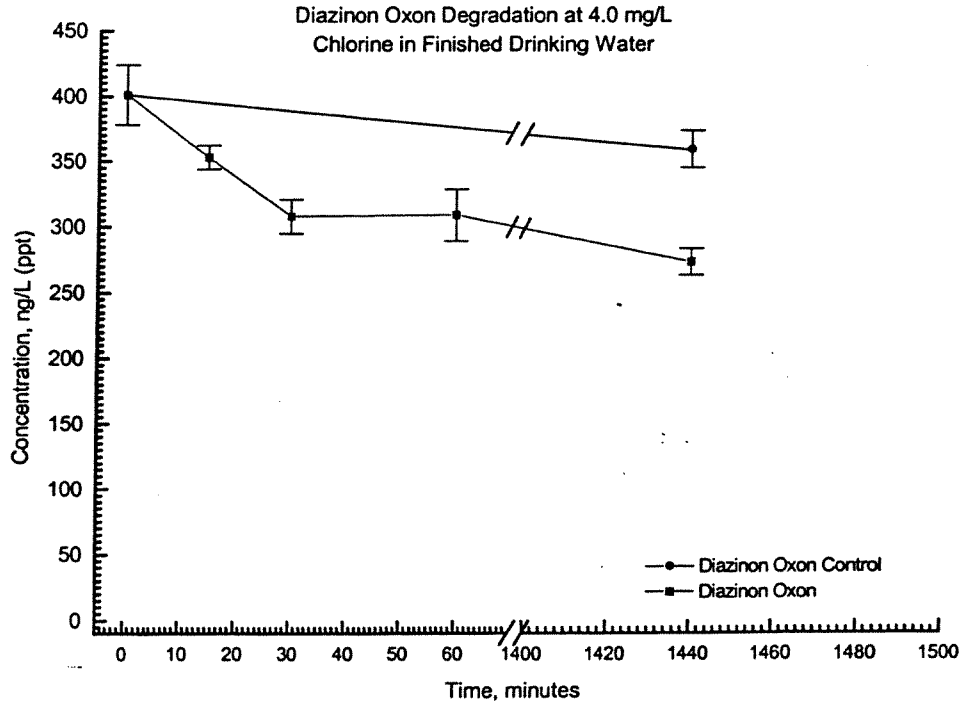


Figure 18
Chlorpyrifos Degradation and Oxon Formation
at 4.0 mg/L Chlorine in Finished Drinking Water

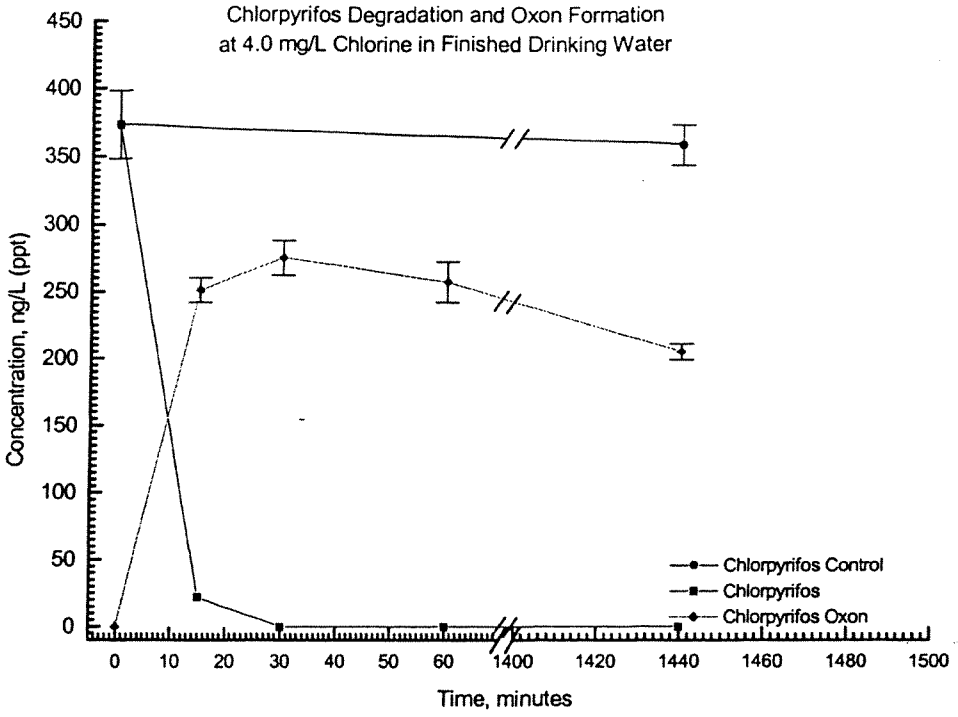


Figure 19
Chlorpyrifos Oxon Degradation at 4.0 mg/L
Chlorine in Finished Drinking Water

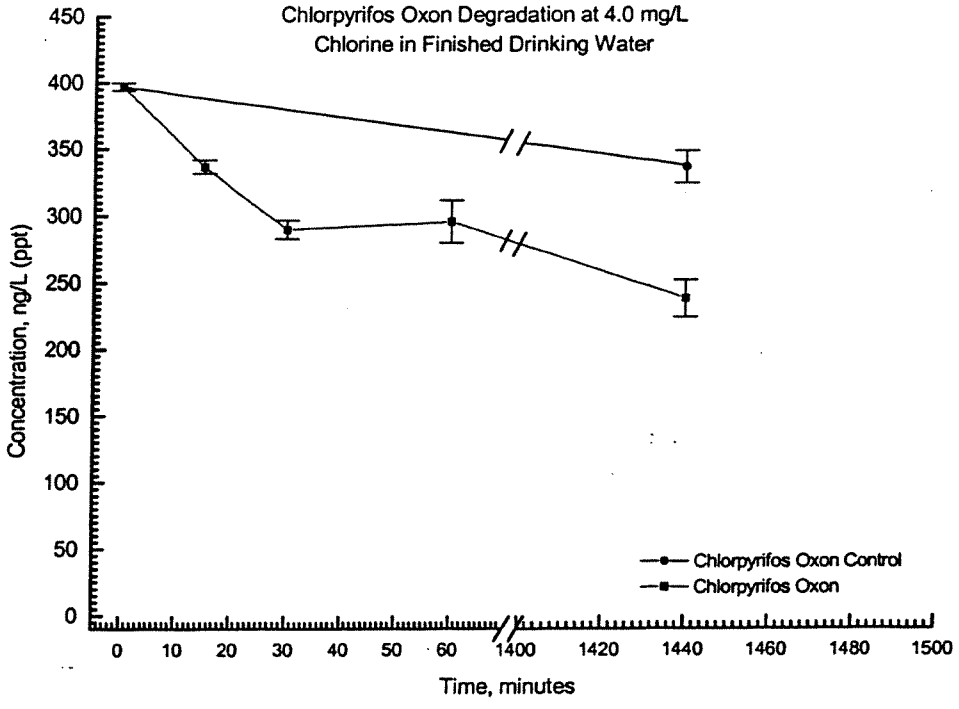


Figure 20
Malathion Degradation and Oxon Formation at
4.0 mg/L Chlorine in Finished Drinking Water

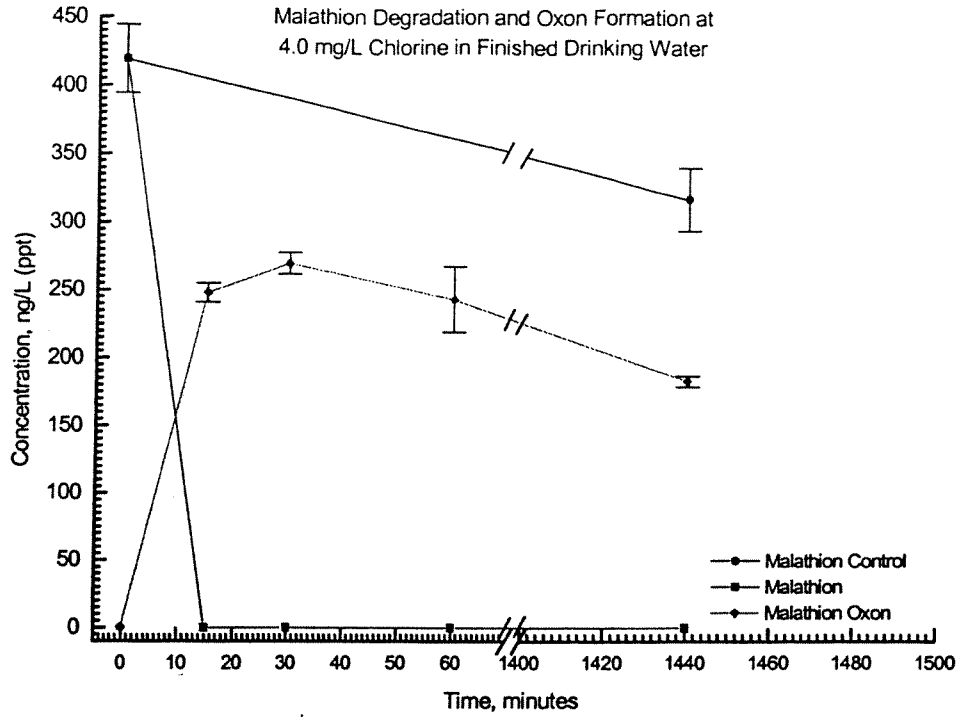


Figure 21
Malathion Oxon Degradation at 4.0 mg/L
Chlorine in Finished Drinking Water

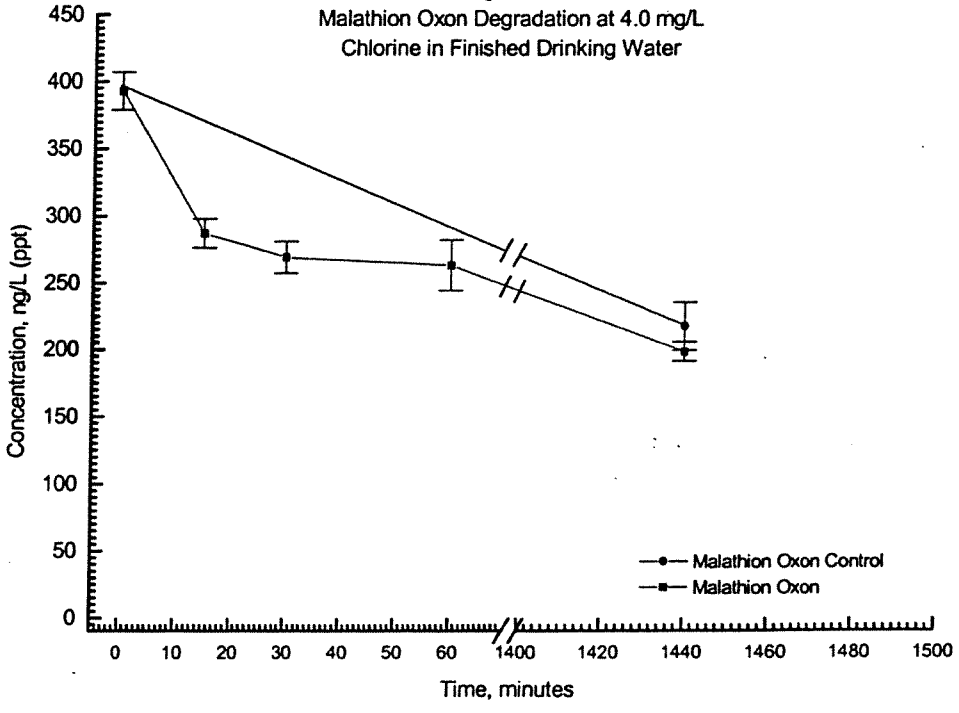


Figure 22
Azinphos Methyl Degradation and Oxon Formation at 4.0 mg/L
Chlorine in Finished Drinking Water

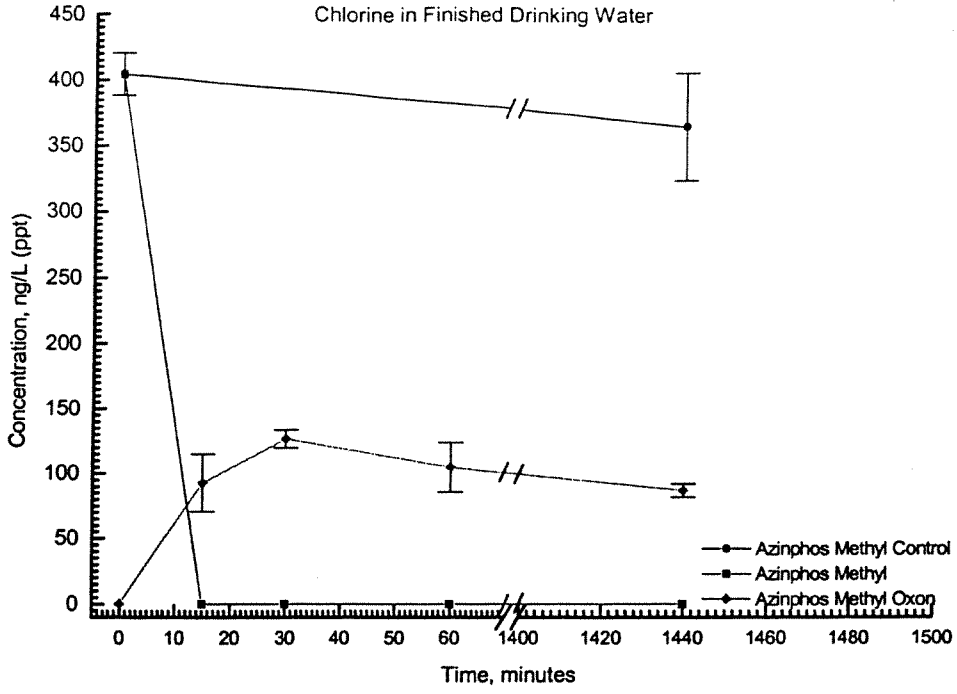


Figure 23
Azinphos Methyl Oxon Degradation at 4.0 mg/L
Chlorine in Finished Drinking Water

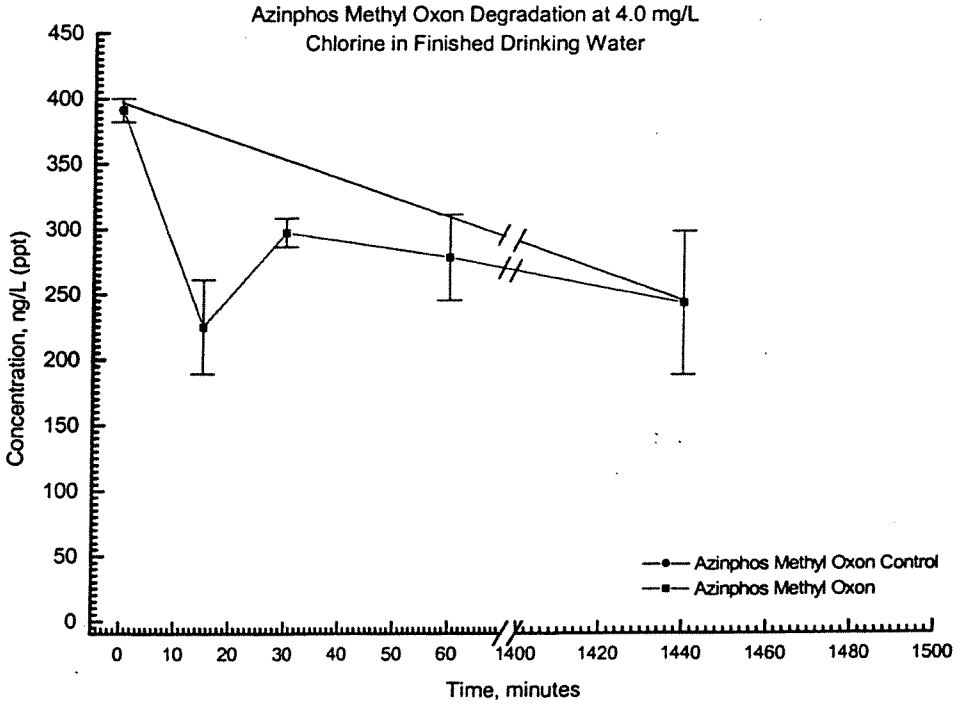


FIGURE 24
OXON DEGREDATION IN CONTROL VERSUS EXPERIMENTAL SAMPLES AFTER 24 HOURS, 4 mg/l CHLORINE CONCENTRATION

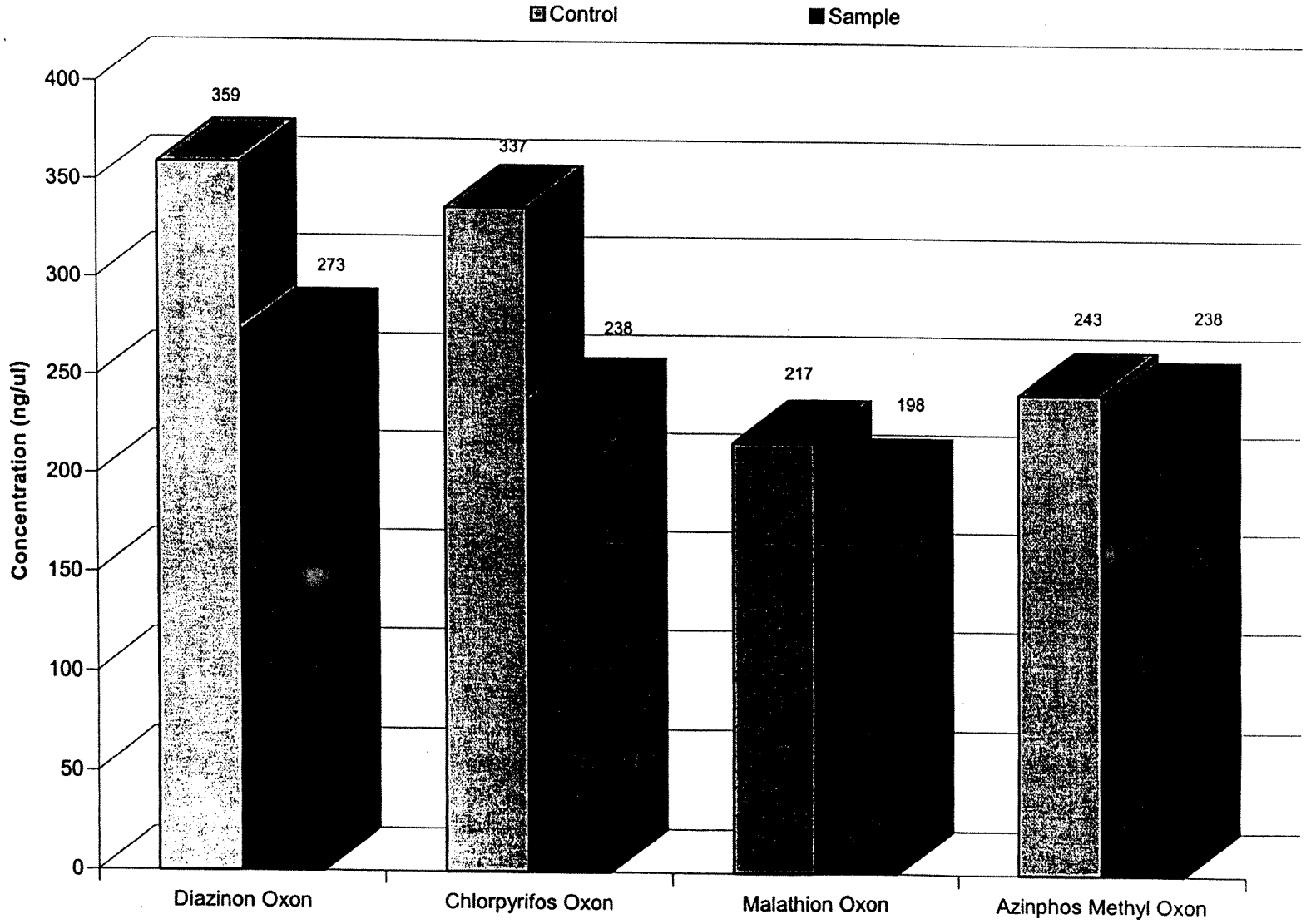


FIGURE 25
24HR CONTROL SAMPLES, ACEPHATE AND METHAMIDOPHOS

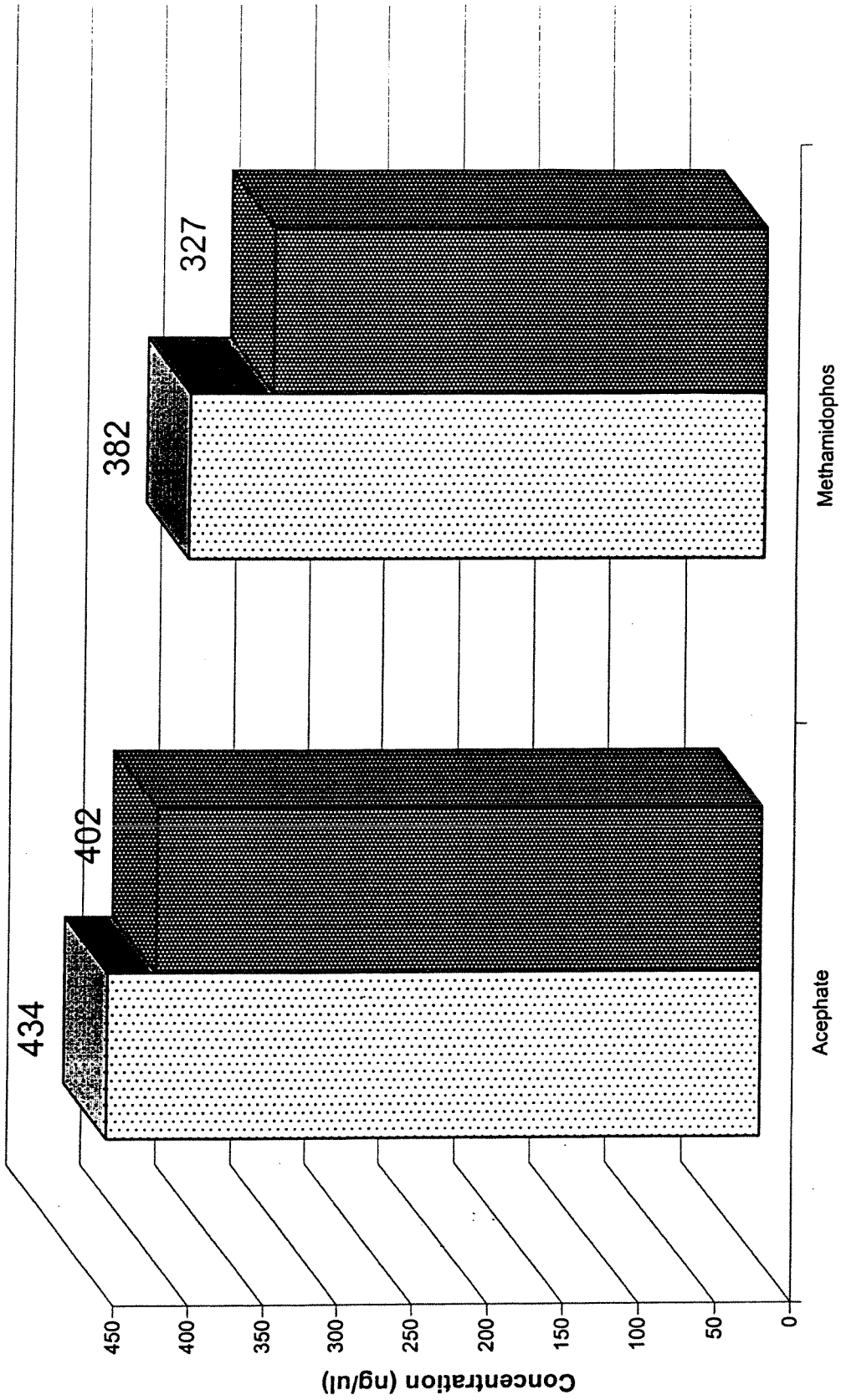


FIGURE 26
DEGRADATION OF ACEPHATE AND FORMATION OF METHAMIDOPHOS
AT 2 mg/L CHLORINE CONCENTRATION (% REMAINING)

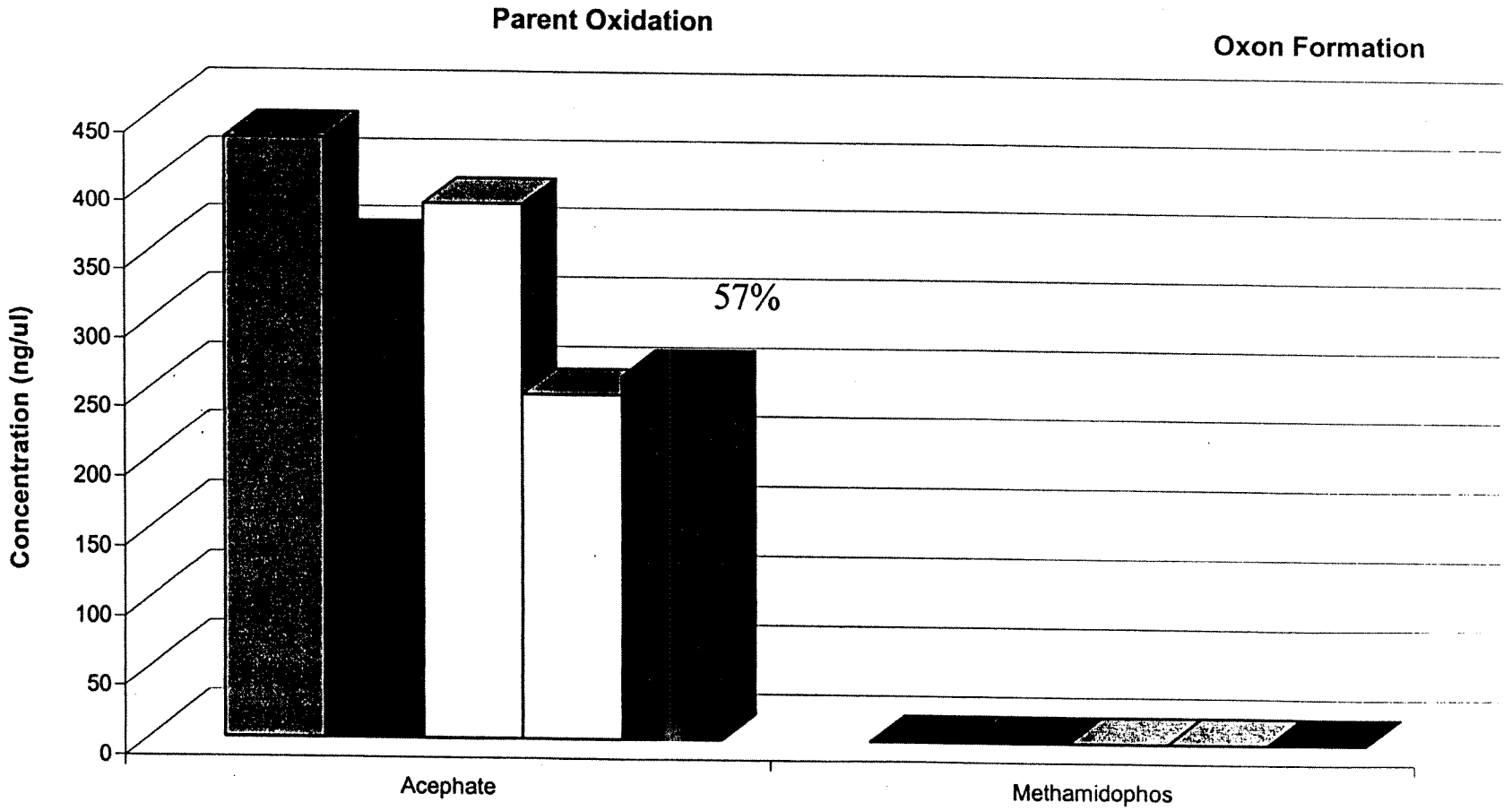


FIGURE 27
DEGRADATION OF METHAMIDOPHOS AT 2 mg/L CHLORINE CONCENTRATION
(% REMAINING)

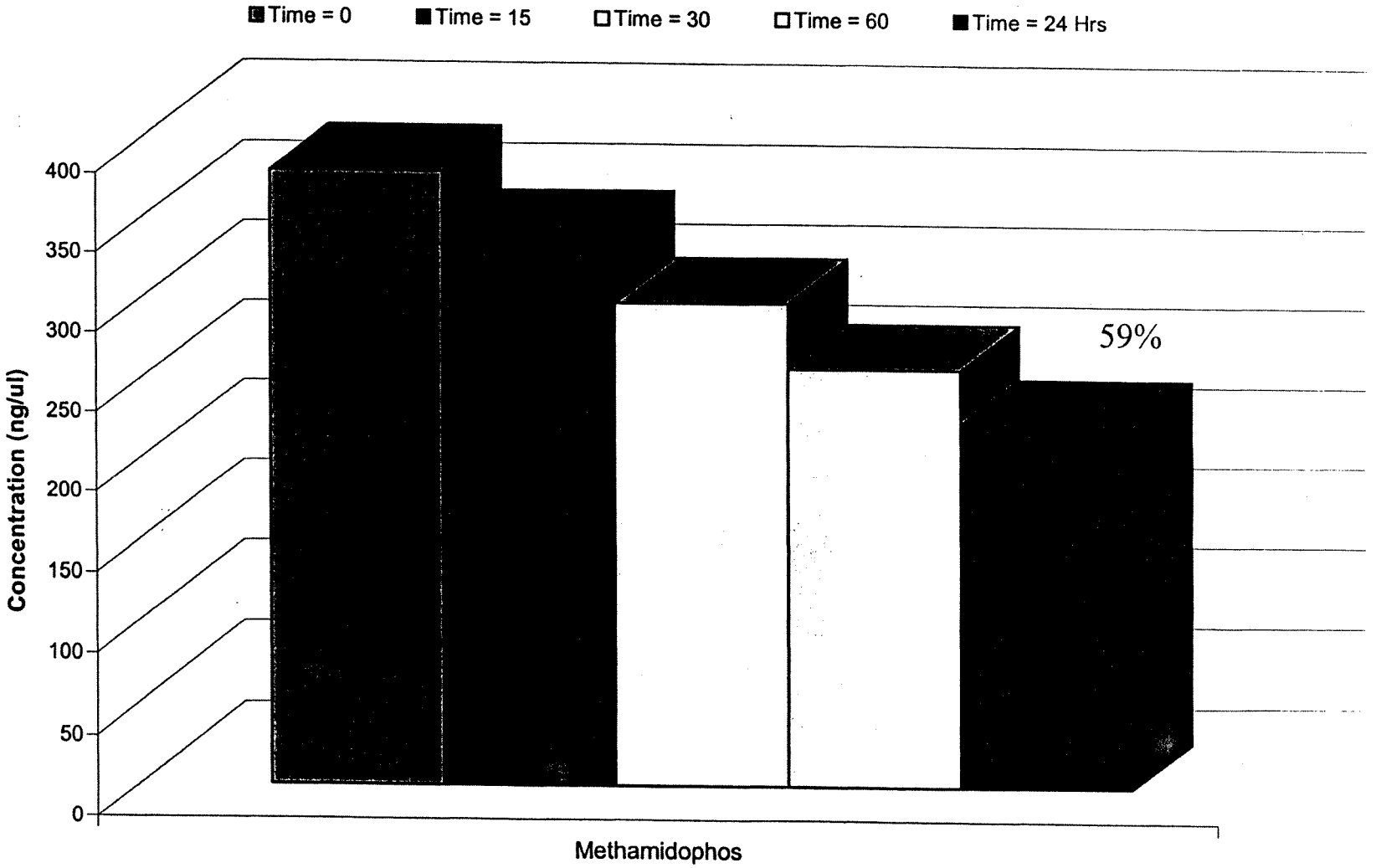


Figure 28
Acephate Degradation and Methamidophos Formation at 2.0 mg/L Chlorine in Finished Drinking Water

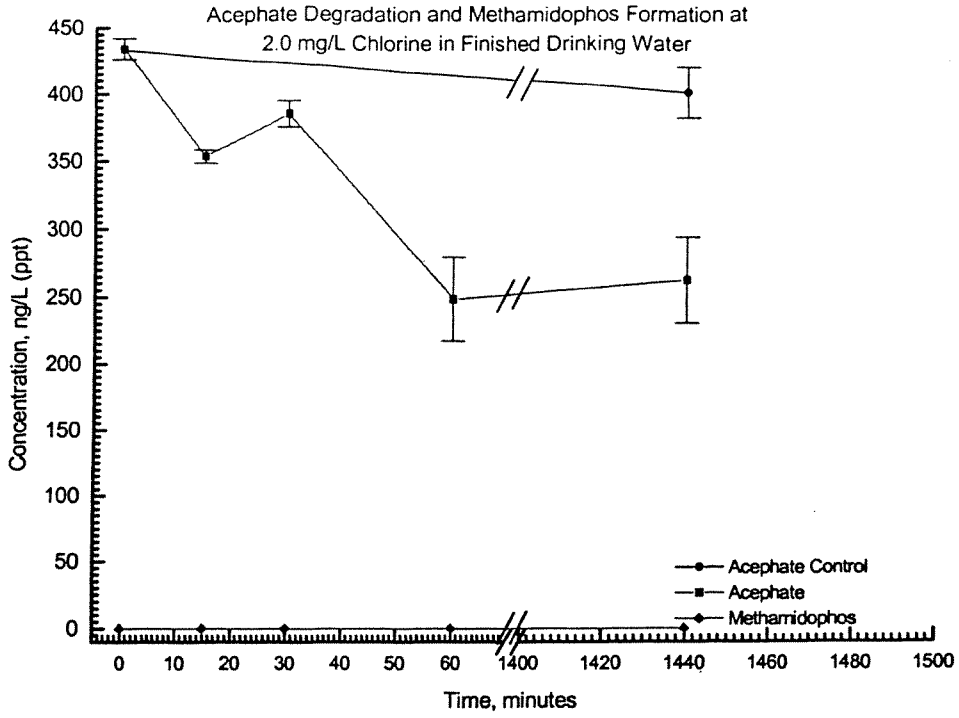


Figure 29
Methamidophos Degradation at 2.0 mg/L Chlorine in Finished Drinking Water

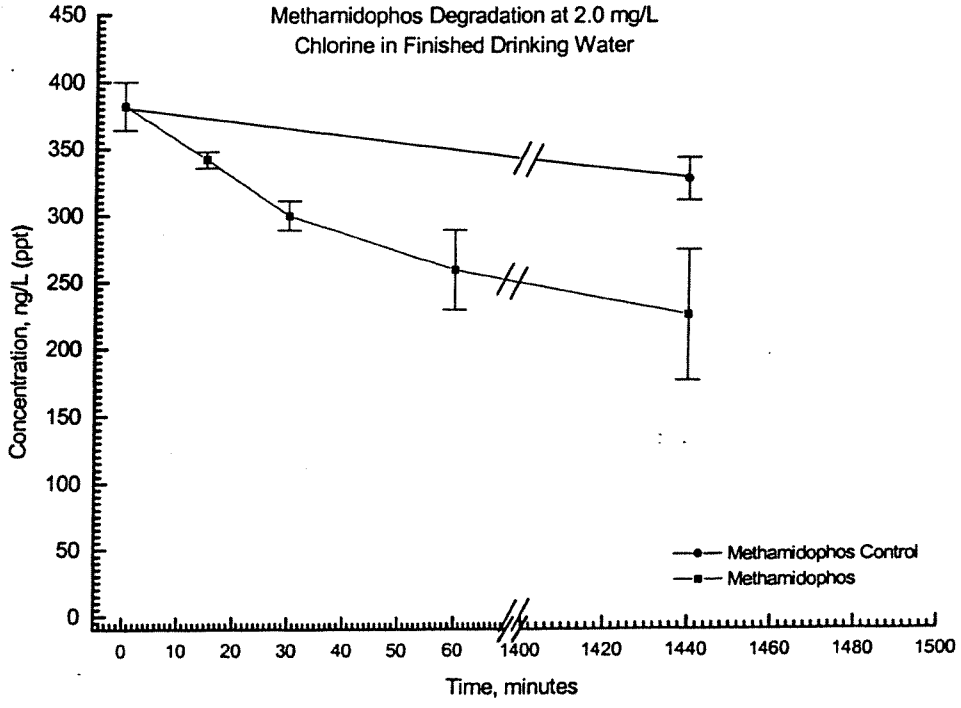


FIGURE 30
DEGRADATION OF ACEPHATE AND FORMATION OF METHAMIDOPHOS
AT 4 mg/L CHLORINE CONCENTRATION (% REMAINING)

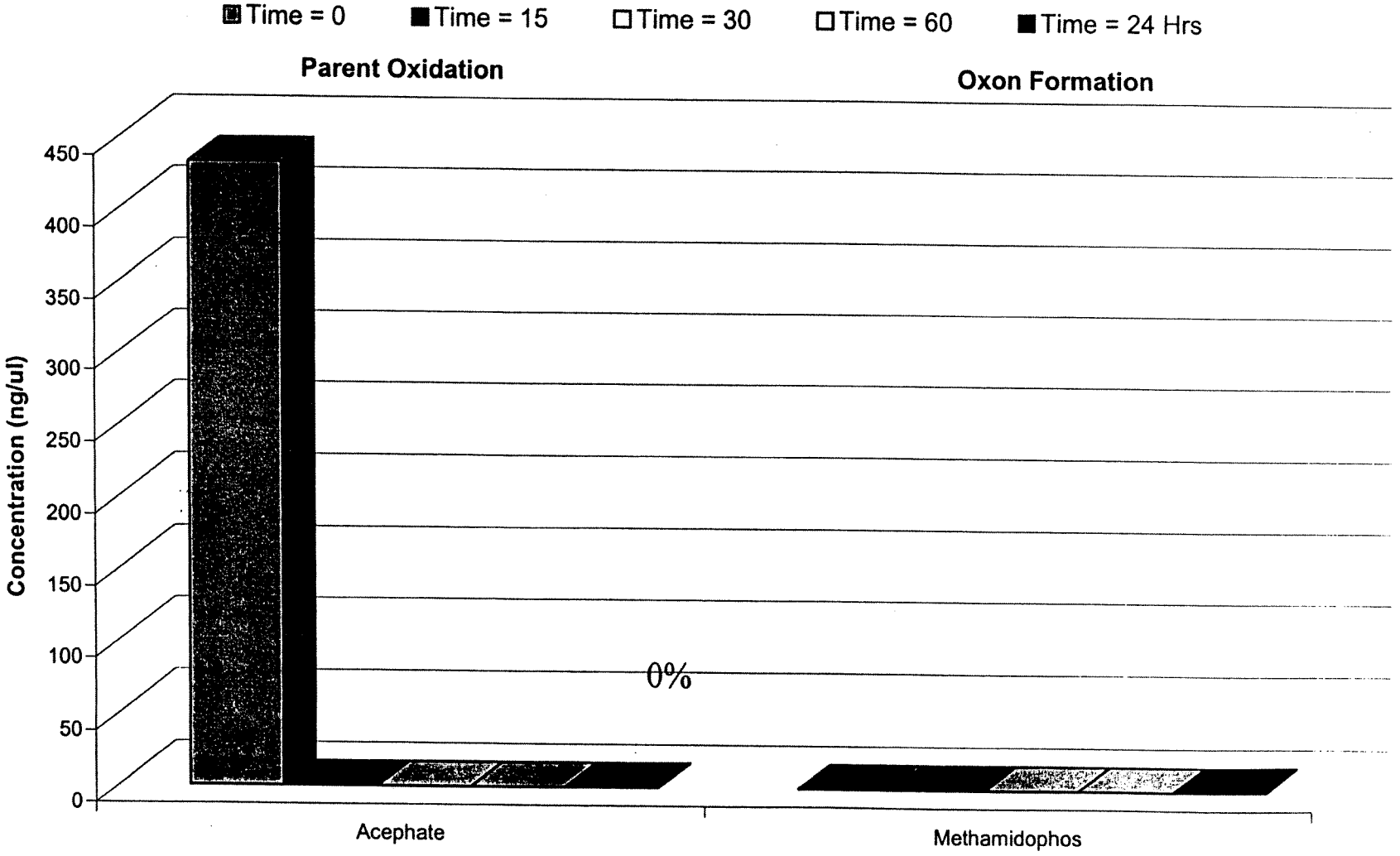


FIGURE 31
DEGRADATION OF METHAMIDOPHOS AT 4 mg/L CHLORINE CONCENTRATION
(% REMAINING)

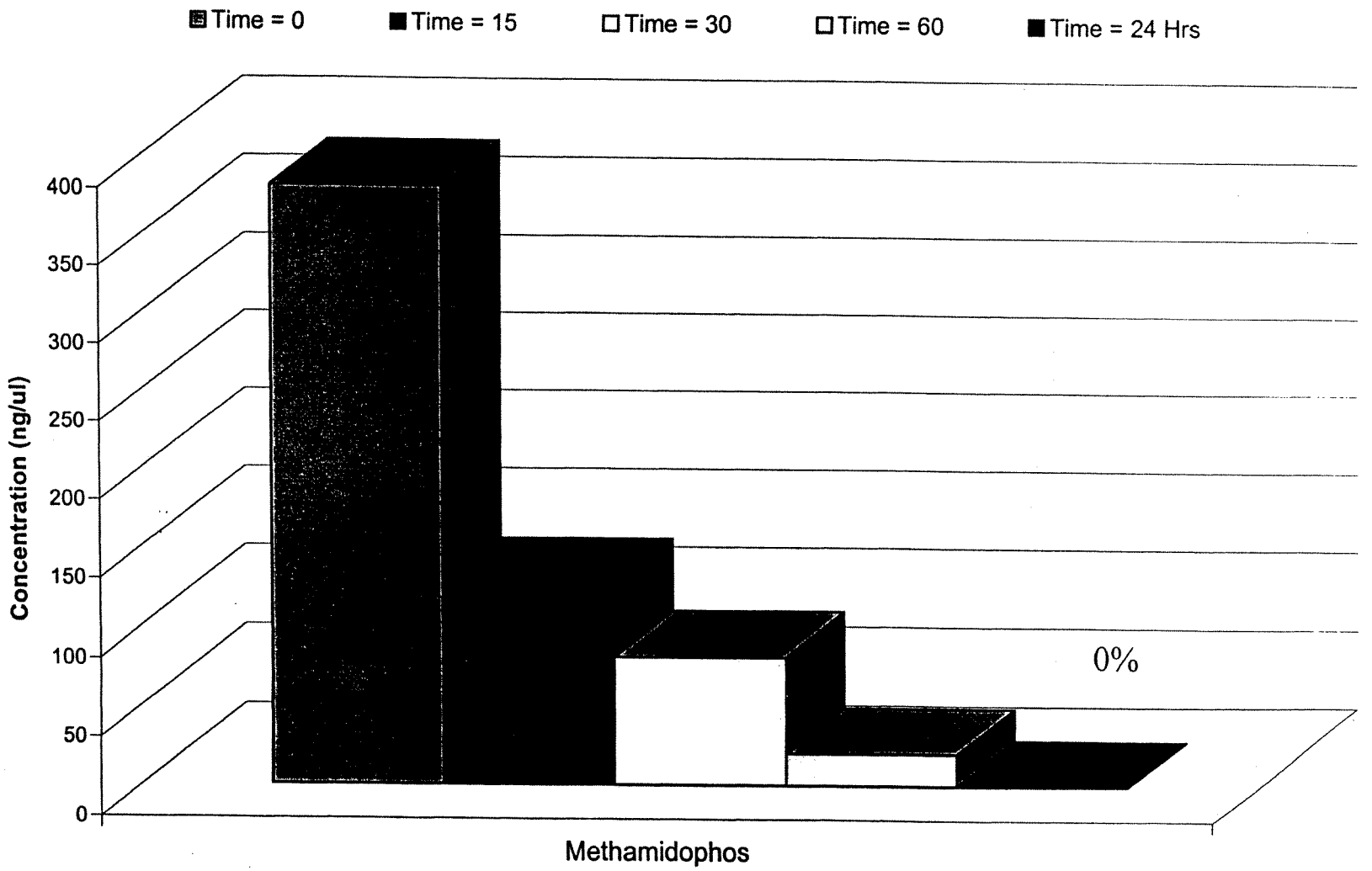


Figure 32
Acephate Degradation and Methamidophos Formation at 4.0 mg/L Chlorine in Finished Drinking Water

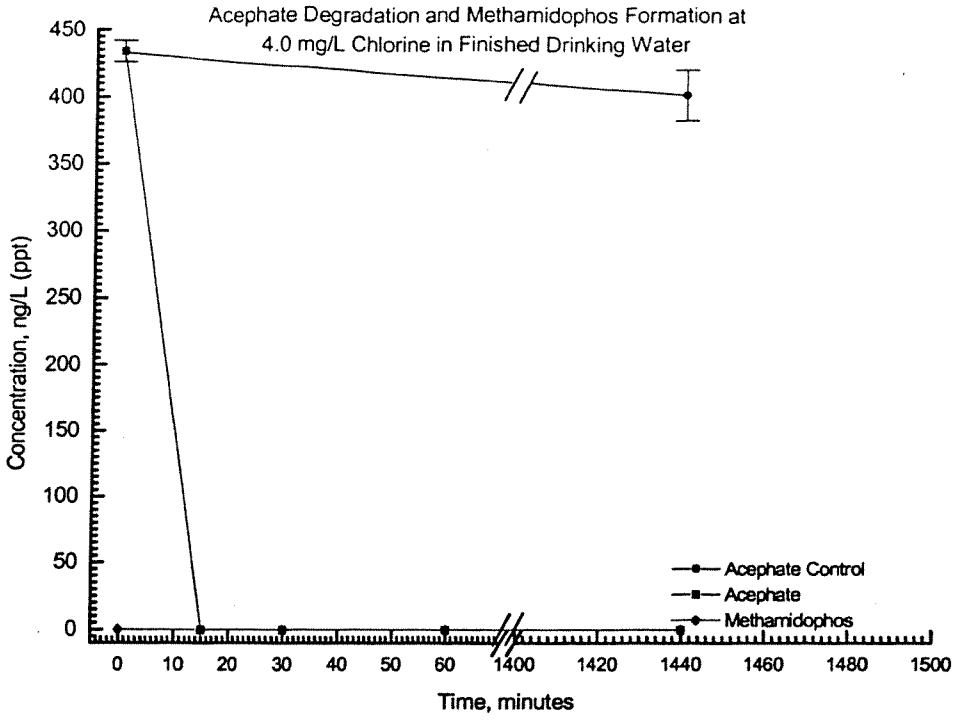
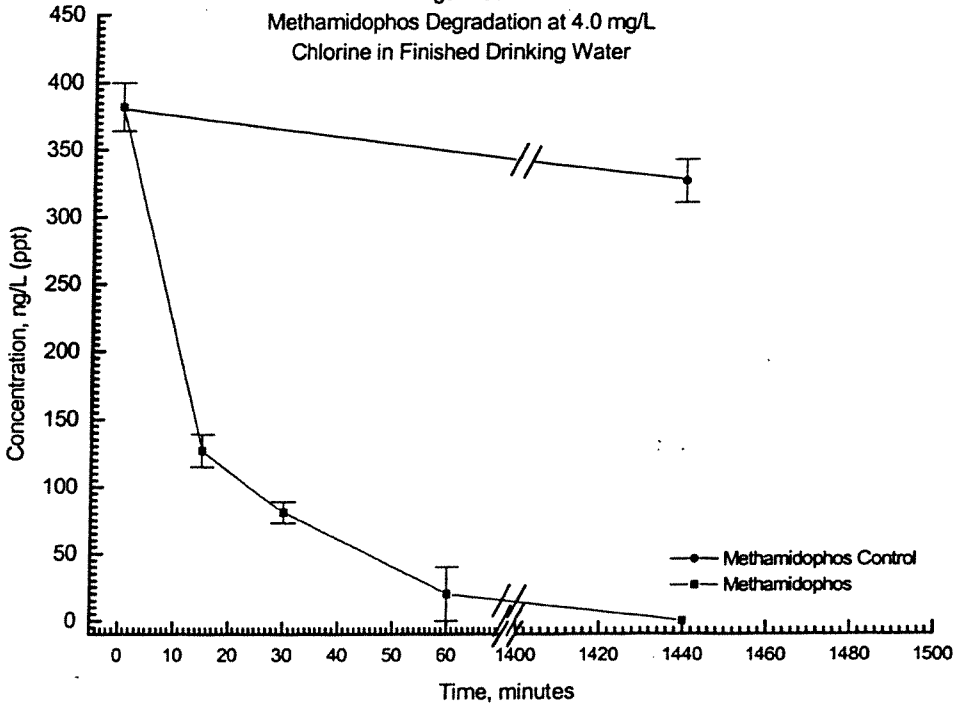


Figure 33
Methamidophos Degradation at 4.0 mg/L Chlorine in Finished Drinking Water



APPENDIX A

ANALYTICAL DATA VALIDATION

Statement of Quality Assurance

Study Title: Chlorine Degradation of Six Organophosphorus Insecticides and Four of Their Oxons in a Drinking Water Matrix

Study No.: *En•fate* Study No. 00102

Audits of this study were conducted as required by Good Laboratory Practice regulations of USEPA, 40 CFR Part 160. It is concluded that the results presented in this report accurately describe the methods and standard procedures followed and reflect the raw data generated during the conduct of the study

Company Agent: _____ Date: 2-15-2000

Wendy Stehling
Quality Assurance Coordinator

Certificate of Good Laboratory Practice

The study described in this document was conducted under GLP and meets the requirements of 40 CFR Part 160.

Performing Lab: Environmental Analytical Solutions, Inc. (EASI)
2501 Lexington Avenue
Kenner, Louisiana 70062

Analytical Study Coordinator :

Date: 7-15-2000

V. C. Culpepper, Sc.D.
Laboratory Manager

Management:

Date: 7-15-2000

Shau-Nong Chang, Ph.D., CIH
President

ANALYTICAL DATA VALIDATION:

C-18 SOLID PHASE EXTRACTION AND ANALYSIS OF EXTRACTS FOR DIAZINON, DIAZINON OXON, CHLORPYRIFOS, CHLORPYRIFOS OXON, MALATHION, MALATHION OXON, GUTHION, AND GUTHION OXON BY GC/MS IN SELECTED ION MODE

Analytical data is summarized in Tables 1-4 and included in Appendix B. Analysis of the sample extracts was performed during two run sequences. All aqueous samples were extracted within 48 hours of collection. Extracts were analyzed outside of the recommended 40-day holding time. However, studies have been conducted confirming the stability of the target analytes in organic extracts at storage times exceeding 100 days.

Sequence Name: HPCHEM\1\SEQUENCE\081099.S

Target analytes were not detected in the method and matrix blanks. Target analytes were not detected in the finished drinking water control sample. Average recoveries of matrix spike/matrix spike duplicate analyses were greater than 70% for all analytes except chlorpyrifos oxon, where the average recovery was 64%. Precision between matrix spike/matrix spike duplicate analyses was 10% or less for all analytes.

Five calibration check standards were analyzed throughout the run sequence, after every 10 sample analyses and at the end of the run sequence. The response of the analytes was within +/- 20% of the initial calibration response with the exception of guthion oxon in the last three check standards. The response of guthion oxon progressively diminished during the run sequence to a level approximately 32% less than the initial calibration response. This may be due to the decreased recovery of guthion oxon in the three replicates from the 2 ppm chlorine oxidation experiment, t = 15 minutes.

With the exception of the above three replicates, the quality control data supports the accuracy and usability of the data for the experiment.

Sequence Name: HPCHEM\1\SEQUENCE\081299.S

Target analytes were not detected in the method and matrix blanks. Target analytes were not detected in the finished drinking water control sample. Average recoveries of matrix spike/matrix spike duplicate analyses ranged from 67% to 69% for all analytes except chlorpyrifos oxon, where the average recovery was 55%. Precision between matrix spike/matrix spike duplicate analyses was 10% or less for all analytes. The lower spike recoveries do not affect data quantification. For example, two replicates from the 4 ppm chlorine oxidation experiment, t = 15 minutes, were analyzed during this run sequence and one replicate was analyzed during run sequence 081099.S. The precision of the replicates was 4% or less for all analytes detected with the exception of guthion oxon (cv=24%). The guthion oxon concentration was significantly lower on the replicate analyzed during run sequence 081099.S due to reasons previously stated.

Three calibration check standards were analyzed throughout the run sequence, after every 10 sample analyses and at the end of the run sequence. The response of the analytes was within +/- 20% of the initial calibration response for all three check standards.

The quality control data supports the accuracy and usability of the data for the experiment.

Target analytes were not detected in the method and matrix blanks. Target analytes were not detected in the finished drinking water control sample. Average recoveries of matrix spike/matrix spike duplicate analyses were greater than 70% for all analytes except chlorpyrifos oxon, where the average recovery was 64%. Precision between matrix spike/matrix spike duplicate analyses was 10% or less for all analytes.

Five calibration check standards were analyzed throughout the run sequence, after every 10 sample analyses and at the end of the run sequence. The response of the analytes was within +/- 20% of the initial calibration response with the exception of guthion oxon in the last three check standards. The response of guthion oxon progressively diminished during the run sequence to a level approximately 32% less than the initial calibration response. This may be due to the decreased recovery of guthion oxon in the three replicates from the 2 ppm chlorine oxidation experiment, t = 15 minutes.

With the exception of the above three replicates, the quality control data supports the accuracy and usability of the data for the experiment.

AC-2 SOLID PHASE EXTRACTION AND ANALYSIS OF EXTRACTS FOR ACEPHATE AND METHAMIDOPHOS BY GC/FPD.

Analytical data is summarized in Tables 5 and 6 and included in Appendix B. Analysis of the sample extracts was performed during two run sequences. All aqueous samples were extracted within 48 hours of collection. Extracts were analyzed outside of the recommended 40-day holding time. However, studies have been conducted confirming the stability of the target analytes in organic extracts at storage times exceeding 100 days.

Sequence Name: 080800

Target analytes were not detected in the matrix blanks, which also served as the control samples. Average recoveries of matrix spike/matrix spike duplicate analyses were greater than 70% for acephate . The average recovery of matrix spike pairs were less than 70% for methamidophos (52% and 59%). The precision between matrix spike replicates high for both analytes. Similar recovery and precision problems were noted with other related studies. The low recovery and poor precision of the matrix spikes did not reflect the analytical data or affect sample quantification. Overall the precision of the replicates at each time interval was significantly better than the matrix spikes. Additionally, the recovery of the target analytes, as measured by the T=0 control samples, was high as well. The average recovery of the acephate was 87% (cv = 8%) and the average recovery of

methamidophos was 76% (cv = 18%). Acephate and methamidophos recoveries in the t=24 hour control samples were 80% (cv = 19%) and 65% (cv = 16%) respectively.

Five calibration check standards were analyzed throughout the run sequence, after every 10 sample analyses and at the end of the run sequence. The response of the analytes was within +/- 20% of the initial calibration response.

The quality control data supports the usability of the data for the experiment.

Sequence Name: 080900

Target analytes were not detected in the matrix blanks, which also served as the control samples. The average recovery of the matrix spike/matrix spike duplicate was greater than 70% for both compounds.

Four calibration check standards were analyzed throughout the run sequence, after every 10 sample analyses and at the end of the run sequence. The response of acephate was 39% higher and methamidophos 24% higher than the initial calibration response in the first standard. In the second check standard, the response of acephate was 25% higher than the initial calibration, while methamidophos was within 20%. Acephate and methamidophos were within 20% of the initial calibration response in the other two check standards. Acephate data was not affected by the higher response. At a 4 ppm chlorine concentration the acephate was oxidized to a non-detectable level in all samples. Methamidophos response was only slighter than 20% in the first check standard and within 20% in the succeeding check standards. Methamidophos quantification may be slightly higher than actual, however; the degree is probably insignificant.

The quality control data supports the accuracy and usability of the data for the experiment.

APPENDIX B

PROTOCOL, AMENDMENTS AND DEVIATION

TABLE OF CONTENTS
APPENDIX B

COPY

STUDY PROTOCOL

CHLORINE DEGRADATION OF SELECTED ORGANOPHOSPHORUS PESTICIDES AND CERTAIN OF THEIR DEGRADATES IN A DRINKING WATER MATRIX

Data Requirement

EPA Food Quality Protection Act

Sponsors

Novartis Crop Protection, Inc.
410 Swing Road
Greensboro, NC 27409

Bayer Corporation
17745 South Metcalf
Stillwell, KS 66085

Dow Agrosciences, LLC
9330 Zionsville Road
Indianapolis, IN 46268

Cheminova Agro A/S
Thybøronvej 76-78
Harboøre, Denmark 7673

Valent U.S.A.
1333N. California Blvd.
Walnut Creek, CA 94596

Study Identification Number

En•fate Study No. 00102

Testing Facility

Novartis Crop Protection
410 Swing Road
Greensboro, NC 27409

Total Pages: 50

PRINCIPAL STUDY PERSONNEL

Study Director	Dennis Tierney, Ph.D. Novartis Crop Protection, Inc. 410 Swing Road Greensboro, NC 27409 Tel: 336-632-2850 Fax: 336-632-2290
Study Coordinator	Brian R. Christensen *En•fate, LLC* 14280A 23rd Ave N Plymouth, MN 55447 Tel: (612) 559-9101 Fax: (612) 559-9184

PROTOCOL APPROVAL

Title: Chlorine Degradation of Selected Organophosphorus Pesticides and Certain of their Degradates in a Drinking Water Matrix

En•fate Study No.: 00102

En•fate Project No.: 007.3

Proposed Experimental Start Date: June 7, 1999

Proposed Experimental Termination Date: July 30, 2000

Study Coordinator, *En•fate, LLC*

Brian R. Christensen
Principal Scientist

6·3·99
Date

Study Director, Novartis Crop Protection

Dennis P. Tierney, Ph.D.
Environmental Product Manager
Agriculture Stewardship Group
Department of Science

6-5-99
Date

Management, Novartis Crop Protection, Inc. and Sponsor Representative

Janis McFarland, Ph.D.
Director
Agriculture Stewardship Group
Department of Science

6-4-99
Date

Quality Assurance, Novartis Crop Protection, Inc

Tom Gale, Jr.
Manager
Quality Assurance Unit

6-4-99
Date

TABLE OF CONTENTS

1.0 INTRODUCTION

In evaluating tolerances for pesticides under the Federal Food, Drug, and Cosmetics Act as amended by the Food Quality Protection Act (FQPA), the Environmental Protection Agency (EPA) is directed to take into account not only food residue levels but also reliable information on certain other types of potential exposure, including drinking water. A concurrent study is being conducted to determine the level, if any, of several organophosphorus pesticides in finished drinking water. Chlorine is frequently used for biological disinfection at water treatment plants. This study will evaluate degradation of selected organophosphorus pesticides and certain of their degradates by chlorine in finished drinking water. It will also evaluate the potential oxidation of organophosphorus pesticides into their degradates and the potential oxidation of the degradates into further degradation products.

This study is sponsored by: Dow Agrosciences, a registrant of products containing chlorpyrifos; Bayer, a registrant and producer of products containing azinphos-methyl and methamidophos; Cheminova Agro A/S, a registrant of products containing malathion; Novartis Crop Protection, Inc., a registrant and producer of products containing diazinon; and Valent, Inc., a registrant of products containing acephate.

This study is being performed in parallel with *En•fate* Study No. 00100 "Community Water System Finished drinking water Monitoring Study for Organophosphorus Pesticides and their Major Degradation Products in the United States".

2.0 STUDY OBJECTIVE

This study will evaluate the effect of total residual chlorine on the integrity of target organophosphorus pesticides and certain of their degradates (hereinafter degradates) at selected contact time intervals. The target pesticides and degradates (in parentheses) include: acephate (methamidophos), diazinon (diazinon oxon), chlorpyrifos (chlorpyrifos oxon), malathion (malathion oxon), and azinphos-methyl (azinophos oxon). The data will determine the effect of chlorine on the analytes and certain of their degradates at chlorine concentrations within the range typically seen in finished finished drinking waters at selected time intervals up to 24 hours.

3.0 TEST SITES

Environmental Analytical Solutions, Inc. (EASI) 2501 Lexington Ave, Kenner, LA 70062 will be the testing site. EASI will perform the analytical tests. Analytical tests and methodologies are described in Section 6 of this Protocol. Analytical results and a final report will be submitted to *En•fate*, LLC.

En•fate, LLC, 14280A 23rd Ave North, Plymouth, MN 55447 will coordinate the performance of the study.

4.0 EXPERIMENTAL METHODS

4.1 Reference Substances

4.1.1 Field Tests

No field tests will be conducted during this study.

4.1.2 Laboratory Tests

Laboratory test substances are: acephate, azinphos methyl, azinphos methyl oxon, chlorpyrifos, chlorpyrifos oxon, diazinon, diazinon oxon, malathion, malathion oxon, and methamidophos.

The reference standards for this study (GLP characterized) are supplied by Novartis Crop Protection, Inc., Greensboro, North Carolina; Bayer Corporation, Agricultural Division, Stillwell, Kansas; Dow Agrosciences, Indianapolis, Indiana; Cheminova Agro A/S, Lemvig, Denmark; and Valent, U.S.A, Dublin, California. Novartis will supply diazinon and its oxon. Bayer will supply azinphos-methyl, its oxon and methamidophos. Cheminova will supply malathion and its oxon. Valent will supply acephate. Dow Agrosciences will supply chlorpyrifos and its oxon. Reference substance identification is provided in Attachment A - Analytical Methodology.

Finished drinking water will be obtained and stored in high density polyethylene containers (HDPE). The water will be analyzed for total residual chlorine using the DPD colorimetric method. Residual chlorine, if present, will be quenched using stochiometric amounts of analytical grade sodium thiosulfate. To ensure target analyte recovery and precision, the water will be fortified with each analyte at a concentration of approximately 0.5 µg/L. The actual concentration will be verified by analysis of post-spike control samples. The water will be equally divided into HDPE containers and fortified with analytical grade sodium hypochlorite at residual chlorine concentrations of 0 mg/L, 2 mg/L, and 4 mg/L (typical of the range at water treatment plants). The water will be portioned to amber glass bottles and held at 20°C. At each contact time interval total residual chlorine will be immediately quenched with excess sodium thiosulfate. Samples will be chilled to approximately 4oC prior to extraction. Samples will be extracted and analyzed in triplicate for each sampling event.

A replicate of the above procedure will be performed using oxons of the parent organophosphorus pesticides.

4.2 Test System Identification and Justification

The test system is finished drinking water from the Jefferson Parish Louisiana Water Treatment Plant. The plant servicing the EASI laboratory facility was selected. Process operations at this facility for supplying finished drinking water are similar to most water treatment plants in the United States supplying finished drinking water from surface water sources.

4.3 Sampling Schedule and Sample Collection

Three replicates will be extracted and analyzed at the following time intervals: 15, 30 and 60 minutes, and 24 hours at initial total residual chlorine concentrations of 2 and 4 mg/L. Control samples (0 mg/L chlorine) will be sampled in triplicate at 0 and 24 hours. Samples will be identified by EASI sequential GLP log number, the date, and replication number.

4.4 Bias

For each set of 20 samples, matrix spike/matrix spike duplicate samples will be extracted and analyzed to monitor for extraction and analytical precision and bias.

5.0 QUALITY ASSURANCE/QUALITY CONTROL

The analytical methods for analyses of parent OP pesticides and certain of their degradation products has been validated (EASI May, 1999). Each analytical set will contain fortification (matrix spikes and matrix spike duplicates) analyzed concurrently for validation of the analyses.

Novartis Crop Protection Quality Assurance Unit (Novartis QAU) will conduct and in-progress inspection. Novartis QAU will also provide an audit of the final report. EASI will perform a data audit and sample verification.

6.0 ANALYTICAL METHODOLOGY

Attachment A contains the analytical methods for this study.

7.0 PROPOSED STATISTICAL METHODS

The following statistical analyses will be conducted from the analytical data in this study:

- Averaging of replicate samples.
- Standard deviation

8.0 REPORTING

The study report will include the results for each pesticide. The study report will meet EPA's formatting requirements as given in PR-notice 86-5. This study report will include, as a minimum:

- A quality assurance statement
- A good laboratory practice statement

- Executive summary
- Introduction
- Description of all analytical methods
- Calculation and statistical methods
- Results and discussion
- GLP compliance statement

9.0 RECORDS TO BE MAINTAINED

All analytical raw data from the above tests including verified copies of the notebook pages will be archived in the Crop Protection Archives located at Novartis in Greensboro, NC, along with the, protocol, protocol amendments (if needed), and final report. Any deviations from this protocol will be documented in a protocol amendment.

Non-study specific data, such as logbooks, will be archived in the Crop Protection Archives located at Novartis, Greensboro, NC after the books are complete.

10.0 GLP COMPLIANCE

All study participants are committed to performing research studies in compliance with current EPA Good Laboratory Practice (GLP) standards. All applicable GLP requirements will be addressed in the execution of this study. The final report will be audited by Novartis QAU and a signed Quality Assurance Statement which includes the dates on which study audits and/or inspections were conducted and reported to study management will be issued for inclusion into the final report.

Any changes to this protocol will be documented in a protocol amendment stating changes made and the reasons for the changes, signed and dated by the Study Director. The Study director will be notified with 24 hours of any protocol deviation.

ATTACHMENT A

ORGANOPHOSPHORUS PESTICIDES

References: USGS Open-File Report 95-181 "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Determination of Pesticides in Water by C-18 Solid-Phase Extraction and Capillary-Column Gas Chromatography/Mass Spectrometry with Selected-Ion Monitoring", 1995

US EPA Test Methods for Evaluating Solid Waste, SW-846, 3rd edition, Method 8141A.

US EPA 40 CFR Part 136, Appendix B. "Definition and Procedure for the Determination of the Method Detection Limit"

US EPA Method 1618: Organo-halide Pesticides, Organo-phosphorus Pesticides, and Phenoxy-acid Herbicides by Wide Bore Capillary column Gas Chromatography with selective Detectors. July 1989.

Holding Time: All samples must be extracted within 7 days of collection and completely analyzed within 30 days of extraction. Disposal of samples will be only with the approval of the study director.

Preservation: Sample container must contain sodium thiosulfate at 0.01% to quench the redox potential of any residual chlorine or chloramine that may be added by a community water system. All samples must be protected from light and refrigerated at 4°C ± 2°C from the time of collection until extraction.

Sampling: For water samples, 1 L of water is required for extraction and should be collected in sufficient volume for a second analysis, i.e. ≥ 2 liters. Samples must be collected in amber glass containers.

1.0 Scope and application

This method covers the determination of several organophosphorus pesticides and their degradates. This SOP covers sample preparation and analysis. The analytical method was designed to analyze water samples for the presence of organophosphorus pesticides and their primary degradates.

The following compounds (target analytes) are determined by this method:

COMPOUND	CAS No.[a]	MDL(ppb)[b]	PQL(ppb)[b]
Acephate	30560-19-1	0.0320	0.058
Azinphos Methyl	86-50-0	0.0100	0.050
Azinphos methyl oxon	961-22-8	0.0131	0.065
Chlorpyrifos	2921-88-2	0.0089	0.044
Chlorpyrifos oxon	5598-15-2	0.0070	0.035
Diazinon	333-41-5	0.0058	0.029
Diazinon oxon	962-58-3	0.0088	0.044
Malathion	121-75-5	0.0086	0.043
Malathion oxon	1634-78-2	0.0085	0.042
Methamidophos	10265-92-6	0.0170	0.039

[a]: Chemical Abstracts Service Number
[b]: Method Detection Limit and Practical Quantitation Limit as determined by the laboratory upon spiking drinking water from a local treatment facility.

Detection limits of this method are dependent upon the levels of interferences and instrumental limitations. The limits in the table above typify the minimum quantities that can be detected in water treatment facility effluents. The practical quantitation limit (PQL) is generally accepted as 5 times the MDL. The MDL is determined by multiplying the standard deviation of ≥ 7 analyses by the student t value appropriate for that number of analyses (n-1) at 99% confidence level.

2.0 Summary of Method

Solid phase extraction procedures are employed for aqueous samples. Analysis is accomplished by injection of a fixed volume of an extract onto a gas chromatographic column equipped with a fused silica capillary column and detection using a mass selective detector in the selected ion mode.

3.0 Interferences

Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing hardware that lead to discrete artifacts and/or elevated baseline in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of analysis by running laboratory reagent blanks.

All reagents are to be tested prior to use to ensure that interferences do not affect analyses.

4.0 Apparatus and Materials

Gas Chromatograph - Hewlett Packard 5890 Series II.

Autosampler, autoinjector - Hewlett Packard 7673.

Mass spectrometer data system

Mass Selective Detector HP 5971A operated in selected ion mode.

Column
Restek-Rtx-1701 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness Restek # 12023 or equivalent

Alternate Column
Restek - Rtx-200 30 m length x 0.25 mm inner diameter x 0.50 µm film thickness Restek # 15038 or equivalent

Glass powder funnels

Nitrogen evaporation device

Rotary evaporator Buchi Model # R-3000 or equivalent

Autosampler vials

Teflon lined crimp top seals

Vacuum manifold for eluting multiple disks - Baker Speedisk 47 mm Baker # 8095-06 or equivalent

Vacuum or peristaltic pump manifold for eluting multiple cartridges - Baker # 7018-00 or equivalent

Baker Speedisk C18 SPE disks Baker #8055-06 or equivalent

Waters Sep-Pak Plus AC-2 cartridges Waters Custom # WAT020585 (ref#JJAN20229) or equivalent

4.0 Apparatus and Materials, cont.

SPE Polyethylene Reservoirs, 75 ml Baker # 7120-03 or equivalent

Pyrex glass wool

pH paper - wide range

Pipets, disposable glass - 5.75 inch, 9 inch

Syringes - 10 µl, 25 µl, 50 µl, 100 µl, 250 µl, 500 µl, 5 ml and 10 ml

Graduated cylinders – 1 L and 250 ml capacity, Class A

40 ml precleaned VOA vials

50 ml evaporation flasks with 24/40 joint

Analytical balance - capable of accurately weighing 10 g ± 0.0001g

Method blank - analyte free deionized water to which all reagents are added in the same volumes or proportions as used in sample processing, and is carried through the complete preparation and analytical procedure.

Matrix blank – Laboratory potable water to which all reagents are added in the same volumes or proportions as used in sample processing, and is carried through the complete preparation and analytical procedure.

Matrix spike - a known amount of target analyte is added to a sample. Matrix spikes and matrix spike duplicates are used to define matrix specific accuracy and precision of the complete analytical procedure. In addition, spike recoveries are examined to determine the effects of the sample matrix on compound recovery during extraction and analysis.

Surrogate - a compound that is added to all samples, spikes, and blanks. A surrogate is added prior to sample extraction and is used to evaluate sample preparation, matrix effects, and extraction efficiencies.

5.0 Reagents and Standards

Standard grade chemicals

Acephate - Valent U.S.A. Corporation Lot # AS 40p or equivalent

Azinphos Methyl (Guthion) - Bayer Corporation Lot # K-791 or equivalent

Azinphos Methyl oxon - Bayer Corporation Lot # K-166 or equivalent

Chlorpyrifos - Dow AgroSciences Lot # MM 939593-17 or equivalent

Chlorpyrifos oxon - Dow AgroSciences Lot # GS-33-82:126 or equivalent

Diazinon - Novartis Crop Protection Lot # S97-2127 or equivalent

Diazinon oxon - Novartis Crop Protection Lot # S97-2011or equivalent

Malathion - Cheminova Agro A/S Lot # 324-OSJ-54C or equivalent

Malathion oxon - Cheminova Agro A/S Lot # 270-ABB-09-01 or equivalent

Methamidophos - Bayer Corporation Lot # K-753 or equivalent

Triphenylphosphate - Chem Service # O-921 or equivalent

Tributylphosphate - Chem Service # F2191 or equivalent

Semivolatile GCMS Internal Standard Mix, 2000 ng/ul - Ultra Scientific # ISM-560 or equivalent

Perfluorotributylamine (PFTBA)

Organic free water - carbon filtered, deionized water

Hydrochloric acid 1N - to make add 80 ml to 880 ml of deionized water

Acetone - ACS reagent grade

Methanol - ACS reagent grade

5.0 Reagents and Standards, cont.

Methylene Chloride - ACS reagent grade

Ethyl Acetate - ACS reagent grade

Sodium sulfate – granular, anhydrous, ACS reagent grade. Each lot must be extracted with 1:1 methylene chloride:ethyl acetate and analyzed by GC/MS/SIM to demonstrate that it is free of interference before use. Caution: An open container of sodium sulfate may become contaminated during storage in the laboratory.

Helium carrier gas, ultrapure

Nitrogen gas, for evaporation

Stock standard solutions

The preparation of all standards will be documented in the organics standards logbook. Each entry is to be signed and dated by the analyst. The entry should contain adequate information as to how the standard was prepared and how it should be used. The standards should be labeled using the number of the standard logbook and applicable page number to facilitate traceability as well as a short description of the standard, the expiration date and how the standard should be stored.

Stock standards shall be prepared from analytical standards supplied by and characterized in accordance with FIFRA GLPs by the sponsor. It is the responsibility of the sponsor to maintain adequate documentation that verifies compound purity, concentration and identity. Any test/control/reference substances used in the study must be characterized prior to its use in the study.

The surrogate compounds and internal standard compounds are not characterized in accordance with FIFRA GLPs.

If the standards are prepared in the lab care must be exercised to prevent contamination. Glassware must be scrupulously clean. Use high quality solvents and reference materials assayed at 97% or greater purity.

Prepare stock standards by accurately weighing the neat compound to the nearest 0.0001g. The mass of compound to be weighed is dependent upon the amount of standard available and the

size of the volumetric dilution flask. Prepare standards to provide a final concentration of approximately 10,000 ug/ml. For liquid neat standards, use an appropriate microsyringe for optimal control of standard addition during weighing. Dilute stock standards to volume with acetone. Stocks may be prepared as single components or as mixtures.

Store standards in amber screw top vials with Teflon septa. Store at 2-6°C. Standards may be stored up to 1 year unless the standards show signs of degradation.

Working calibration standards are to be prepared on a monthly basis.

Internal Standards

Begin with a 2000 ng/µl semivolatile GCMS internal standard solution (Ultra Scientific #ISM-560, 2000 ng/ul or equivalent). Prepare a 50 ng/µl working solution by diluting 0.25 ml of the stock to 10.0 ml with ethyl acetate. Each 1.0 ml of sample extract should be spiked with 10.0 µl of internal standard solution immediately prior to analysis. The internal standard compounds used are acenaphthene-d_{10}, phenanthrene-d_{10}, and chrysene-d_{12}.

Surrogate Standards

The surrogates used are tributylphosphate and triphenylphosphate. The surrogate solution is spiked prior to extraction using a 0.20 ug/ml working solution (prepared as described below). Prepare a stock solution by weighing 0.1g of neat compound and dissolving in methylene chloride and bringing to volume in a 10.0 ml volumetric flask. Prepare a 50 µg/ml standard solution by diluting 0.050 ml of this stock solution to 10.0 ml in a volumetric flask with acetone. Prepare a 0.20 µg/ml working solution by diluting 0.10 ml of the 50 µg/ml solution to 25.0 ml in a volumetric flask with acetone. Spike 1.0 ml of a 0.20 µg/ml surrogate solution in acetone into 1 L of sample and matrix spikes. For acephate and methamidophos, prepare the final solution in water instead of acetone and spike only 0.25 ml.

Pesticide Matrix Spike Solution

Prepare a matrix spiking solution by mixing and diluting stock standards as detailed for the surrogate standard to produce a solution of 0.20 µg/ml organophosphorus compounds in acetone. For diazinon, chlorpyrifos, guthion, malathion, and their oxons, spike 1.0 ml of this solution into matrix spikes prior to extraction. For acephate and methamidophos, spike 0.25 ml into an empty flask and allow acetone to evaporate before adding samples.

6.0 Procedures

Calibration of equipment

Mass spectrometer performance evaluation

Tune the mass spectrometer daily using the procedure and software provided by the manufacturer. Parameters in tuning are set to give ± 0.15 atomic mass unit resolution at masses 69, 219, and 414 in the spectrum of perfluorotributylamine. Adjust the electron multiplier to get a minimum area of 2,000,000 counts for mass 69 ion. Manually adjust, if necessary, so that the mass 69 ion has 100 percent abundance, mass 219 ion is 40±20 percent, and mass 414 ion is 6.2 ±5.7 percent relative abundance. Check the mass assignments to ensure accuracy to ± 0.15 atomic mass unit in the spectrum scan and that mass peak widths measured at one-half the peak height range from 0.45 to 0.59 atomic mass unit in the profile report. Generate a tune report.

Initial calibration

Acquire initial calibration data using a new capillary column and freshly prepared calibration solutions. Use these data in subsequent evaluation of the GC/MS performance.

Prior to the analysis of each sample set and every 10 samples thereafter during a series of analyses, analyze and evaluate a calibration solution containing all the selected compounds to ensure that the GC/MS performance is in compliance with all established criteria.

The internal standard compounds used are acenaphthene-d_{10}, phenanthrene-d_{10}, and chrysene-d_{12} due to their similar chromatographic behavior to the compounds of interest.

Prepare calibration standards at a minimum of five concentration levels for each compound of interest. Prepare calibration standards at 0.025, 0.050, 0.10, 0.25, 0.50, 0.75, 1.0 µg/ml. Add 10.0 ul of the 50 ng/ul internal standard working solution to 1.0 ml of each calibration standard.

Analyze each standard and acquire data for each calibration solution by injecting 2 µl of each solution into the GC/MS according to the conditions prescribed in Appendix A.

Tabulate peak areas against concentration for each compound and internal standard.

Calculate the response factor (RF_s) for each compound using the following equation:

$$\frac{A_s * C_{is}}{C_s * A_{is}} = RF_s$$

A_s = area of the sample peak
C_{is} = concentration of the internal standard
A_{is} = area of the internal standard
C_s = concentration of the standard compound

Initial calibration data are acceptable if the correlation coefficient, r, is ≥0.99 for linear and the coefficient of the determination, COD, is ≥ 0.99 for non-linear curves calculated across the working concentration range for each compound or surrogate.

Continuing calibration

Calculate the response factor of each compound in each subsequent standard analysis.

If the response for any analyte varies from the predicted response by more than ± 20 %, a new calibration curve must be prepared for that analyte.

Sample Preparation

Extraction Procedure 1: For azinphos methyl, azinphos methyl oxon, chlorpyrifos, chlorpyrifos oxon, diazinon, diazinon oxon, malathion, malathion oxon

Remove samples from refrigerator and allow to reach ambient temperature.

Measure 1 L of sample into a graduated cylinder. Check pH with pH paper. Record the volume and pH in the logbook.

Assemble filter apparatus using Baker Speedisks C-18 SPE disks.

Preclean the extraction apparatus and disk by adding about 5 ml of methylene chloride. Pull a small amount through the disk with vacuum; turn off the vacuum and allow the disk to soak for about two minutes. Pull the remaining solvent through the disk and allow disk to dry. Note: The vacuum apparatus is set to provide a maximum vacuum pressure between 20-25 mm Hg as measured by the inline pressure gauge. Do not adjust the vacuum pump to provide a vacuum greater than 25 mm Hg.

Repeat precleaning step.

Condition the disk by adding about 5-10 ml of methanol to the reservoir, pulling a small amount through the disk then letting it soak for about one minute. Pull most of the remaining methanol through the disk, leaving a layer of methanol above the surface of the disk. DO NOT ALLOW THE DISK TO GO DRY AT THIS POINT!

Add 5-10 ml of deionized water to the disk and pull through the disk leaving 3-5 mm of water above the surface of the disk.

Add 5 ml of methanol and 1.0 ml of surrogate spiking solution to the sample. Record the amount and lot number of surrogate in the logbook. Pour the water sample into the reservoir, under vacuum, filter as quickly as the vacuum will allow. Drain as much water from the graduated cylinder as possible. Rinse the graduated cylinder once with deionized water and add to the reservoir.

After extraction is complete allow the disk to air dry with vacuum on for at least five minutes.

Remove Speedisk, insert a 40 ml vial for eluate collection, and replace Speedisk.

Add about 3 ml of acetone, draw into filter with vacuum on and allow to soak for one minute. Add 5 ml of methylene chloride:ethyl acetate (1:1) to the reservoir. Draw 2-3 ml of the solvent through the disk then release the vacuum. Allow the remaining solvent to soak the disk for about two minutes then draw remainder through with vacuum.

Repeat twice with two more 5 ml aliquots of methylene chloride:ethyl acetate (1:1).

Dry the eluate by passing through approximately 25-30 grams of anhydrous sodium sulfate contained in a small glass filter funnel.

Concentrate the sample extract using nitrogen blowdown to 1.0 ml. Never allow the sample extract to become completely dry.

Prepare a method blank and matrix blank with each group of samples extracted. A method blank consists of a 1 L volume of laboratory deionized water. A matrix blank consists of a 1 L volume of laboratory potable water. Add approximately 0.1 g of sodium thiosulfate and stir until dissolved. Using a syringe, add 1.0 ml of the 0.20 ug/ml working surrogate solution.

For each sample selected for matrix spike and matrix spike duplicate analyses, measure out two additional 1L aliquots and spike each aliquot with 1.0 ml of matrix spike solution and 1.0 ml of

surrogate solution before continuing with the extraction.

Spike 10 µl of internal standard solution into the sample and transfer to an autosampler vial for analysis.

Extraction Procedure 2: For acephate and methamidophos

Remove samples from refrigerator and allow to reach ambient temperature.

Measure 250 ml of sample into a graduated cylinder. Record the volume in the extraction logbook.

Prepare matrix spikes by adding the 0.25 ml of matrix spike solution to a 250 ml boiling flask. Evaporate the residue by placing the flasks under a vacuum hood and air drying with the hood on. Add the samples designated for matrix spikes to dissolve the residue.

Add 0.25 ml of tributylphosphate surrogate solution to all samples.

Assemble filter apparatus using AC-2 cartridges.

Condition the cartridge by sequentially eluting 5 ml of acetone, 10 ml of deionized water, 20 ml of 1 N HCl, and 10 ml of deionized water. Adjust the flow rate to 2-3 ml/minute. Do not allow the cartridge to go dry at any point.

After the conditioning step, add the sample to the reservoir.

After the sample has eluted, rinse the container with 10 ml of deionized water and add to reservoir.

Allow the cartridge to run dry for 2 min.

Remove the cartridge and invert it.

Connect the cartridge to a glass syringe with a luer adapter using a short piece of Teflon tubing.

Add 10 ml of acetone to the syringe and elute 3 ml in the opposite direction of the sample flow into a glass vial.

Stop the elution and allow the cartridge packing material to soak with acetone for 15 minutes before eluting the remaining volume of acetone.

Repeat the elution step with an additional 10 ml aliquot of acetone.

Add 5 ml of ethyl acetate to the eluate and evaporate to dryness on a rotary evaporator. If residual water is present, add an additional 5 ml of ethyl acetate and 10 ml of acetone and re-evaporate.

Dissolve the residue in 0.5 ml of acetone.

Spike 5 µl of internal standard solution into the sample and transfer to an autosampler vial for analysis.

Prepare a method blank and matrix blank with each group of samples extracted. A method blank consists of a 250 ml volume of laboratory deionized water. A matrix blank consists of a 250 ml volume of laboratory potable water. Add approximately 0.1 g of sodium thiosulfate and stir until dissolved. Using a syringe, add 1.0 ml of the 0.20 ug/ml working surrogate solution.

Sample Analysis

These are recommended parameters for the Rtx-200 column. These parameters may be adjusted to optimize responses as necessary.

GC and Detector Conditions for analysis of acephate, methamidophos and tributylphosphate.

Method PSIM2.M – Appendix A
Initial oven temperature -150 °C
Initial time - 1 minutes
Injection volume - 2 µl
Injector temperature 270 °C
Rate - 8°C/min
Final temperature - 200°C
Final time - 0 minutes
Rate A - 20°C/min
Final temperature A - 290°C
Final time A - 6 min
Total runtime - 21.5 minutes

GC and Detector Conditions for analysis of azinphos methyl, azinphos methyl oxon, chlorpyrifos, chlorpyrifos oxon, diazinon, diazinon oxon, malathion, malathion oxon, triphenylphosphate

Method PSIM.M – Appendix B
Initial oven temperature - 150 °C
Initial time - 2 minutes
Injection volume - 2 µl
Injector temperature 270 °C
Rate - 8 °C/min
Final temperature - 290 °C
Final time - 6 minutes
Total runtime - 25.5 minutes

See Appendix A and B for complete printed methods containing GC/MS-SIM data acquisition conditions. These conditions may be adjusted as necessary. The method files also contain the data quantitation parameters. The method quantitates and prints a quantitation report.

Acquire data for each sample using the appropriate method file, PSIM.M or PSIM2.M.

The retention time of the GC peak of the quantitation ion for the selected compound of interest needs to be within ± 6 seconds of the average retention time for each compound as determined from the initial calibration.

Mass spectral verification for each selected compound is done by comparing the relative integrated abundance values of the two significant ions monitored with relative integrated abundance values obtained from calibration solutions analyzed initially. The relative ratios of the primary and secondary ions need to be within ± 20 % of the ratios obtained on injection of a standard free of interferences.

7.0 Calculation of Results

The software will calculate the solution concentration in ng/µl injected. The concentration of the sample can be calculated manually by

$$\frac{C_i * A_c * 1000}{RF_c * A_i * V} = C$$

C = Concentration in the sample in µg/L
C_i = Concentration of the internal standard in µg/ml
A_c = Area of the quant ion of the selected compound
A_i = Area of the quant ion of the internal standard
V = volume of the sample in ml
Rf_c = relative response factor for the selected compound

Sample results are reported to 3 significant figures. For rounding significant figures, refer to EASI SOP *GE-06.01: Reporting Data as a Final Result.*

The internal standard acenphthene-d_{10} is used to calculate acephate, methamidophos and tributylphosphate. Phenanthrene-d_{10} is used to calculate diazinon, diazinon oxon, malathion, malathion oxon, chlorpyrifos and chlorpyrifos oxon. Chrysene-d_{12} is used to calculate triphenylphosphate, guthion and its oxon.

8.0 QC Requirements

The data files should be quantitated and the instrument run log should be filled in as soon as possible after the analysis is complete. During a batch sequence, the data files are to be queued for quantitation immediately after analysis, and the run log filled in as the sequence is completed.

Gas chromatographic retention times may not shift more than thirty seconds. If this should occur, corrective action may be necessary. Check for system malfunction.

Check for saturation of peaks above the calibration range. Dilute the extract accordingly and reanalyze.

Calculate the percent surrogate recovery for the surrogate compound. Surrogates are used by the laboratory to assess extraction and no criteria have been established.

The maximum holding time before sample extraction is 7 days at 0-4 °C. The maximum holding time for final extracts is 30 days at 0-4 °C.

The analyte specific MDL values is 0.05 for the selected organophosphorus pesticides and their degradates in water.

Method blanks are prepared from deionized water. Matrix blanks are prepared from laboratory potable water. One method blank and one matrix blank is required for every group of 20 samples or each time a group of samples are extracted by the same method whichever is more frequent.

A method blank may not contain more than ½ the PQL for any target compound. When a blank exceeds these limits it is considered to be out of control and the blank and all associated samples must be reextracted. The analyst must locate the source of contamination and corrective actions must be taken before data analysis can be continued.

A matrix spike and duplicate are analyzed in order to evaluate the matrix effect of the sample analysis. Matrix spikes and duplicates must be prepared and analyzed each time a group of samples are extracted. Fortified matrix recoveries and relative percent differences are calculated. Matrix recoveries should be between 70 and 120%. The limit for the relative percent difference between spike and duplicate is 40%.

Mass spectrometer tuning criteria. The minimum area for mass 69 ion is 2,000,000 area counts. The mass of 69 ion should be 100 percent abundance, mass 219 ion is 40±20 percent, and mass 414 ion is 6.2 ±5.7 percent relative abundance. The mass assignments must be ± 0.15 atomic mass unit for each ion. The mass peak widths must be between 0.45 to 0.59 atomic mass unit measured at ½ the peak height.

Compound	Retention Time (minutes)	Quantitation Ion (m/z)	Confirmation Ion 1 (m/z)	Confirmation Ion 2 (m/z)
Acephate	7.58	136	94	137
Azinphos methyl	20.84	160	132	none
Azinphos methyl oxon	20.14	160	132	none
Chlorpyrifos	14.51	197	199	314
Chlorpyrifos oxon	15.05	197	199	298
Diazinon	10.86	137	179	153
Diazinon oxon	11.31	137	273	288
Malathion	15.01	125	127	173
Malathion oxon	14.60	127	195	173
Methamidophos	3.92	94	141	136

Compound	Retention Time (minutes)	Quantitation Ion (m/z)	Confirmation Ion 1 (m/z)	Confirmation Ion 2 (m/z)
Tributylphosphate	7.98	99	none	none
Triphenylphosphate	18.37	326	186	none
Acenaphthene-d_{10}	5.00	164	162	none
Phenanthrene-d_{10}	10.82	188	none	none
Chrysene-d_{12}	18.89	240	none	none

The retention time of the GC peak of the quantitation ion for the selected compound of interest needs to be within ± 6 seconds of the average retention time for each compound as determined from the initial calibration. When identifying target analytes in a study sample, the peak shape and width will be evaluated manually by visual inspection of the extracted ion profile to determine that they are similar to those in the fortified samples.

Initial calibration data are acceptable if the correlation coefficient, r, is ≥0.99 for linear and the coefficient of the determination, COD, is ≥ 0.99 for non-linear curves calculated across the working concentration range for each compound or surrogate.

Non-compliance: Analytical performance criteria stated in this SOP may not always be achievable in study samples even when corrective actions were employed in an attempt to meet SOP requirements. In certain pressing situations such as holding time near expiring or quick turnaround requirements, it may be necessary to sacrifice some criteria and proceed with the analysis. Such a decision is left to the study director and will be reported to the study director or his designate as soon as possible. All deviations from the SOP must be thoroughly documented and reported to the study director. The study director is the only individual who can approve changes to the study and will direct the issuance of a protocol deviation.

9.0 Safety

Standard laboratory safety precautions should be adhered to at all times. This assumes that all samples are hazardous.

The use of hoods, safety glasses, lab coats, and any other appropriate safety gear is necessary.

MSDSs are available for all chemicals used in this procedure and should be referred to by all analysts.

APPENDICES

Appendix A

Method PSIM2.M from Chemstation

Method Information For: C:\HPCHEM\1\METHODS\PSIM2.M

Method Sections To Run:

( ) Save Copy of Method With Data
( ) Pre-Run Cmd/Macro =
(X) Data Acquisition
(X) Data Analysis
( ) Post-Run Cmd/Macro =

Method Comments:
This is the SIM method for Acephate and Methamidophos.

END OF TOPLEVEL PARAMETERS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet: GC
Injection Source: GC ALS
Mass Spectrometer: Enabled

HP GC Injector

Front Injector:
No parameters specified

Back Injector:

Sample Washes	1	
Sample Pumps	4	
Injection Volume	2.0	microliters
Syringe Size	10.0	microliters
On Column	Off	
Nanoliter Adapter	Off	
PostInj Solvent A Washes	3	
PostInj Solvent B Washes	3	
Viscosity Delay	0	seconds
Plunger Speed	Fast	

HP5890 Temperature Parameters

Zone Temperatures: State Setpoint

Method: PSIM2.M Wed Jun 02 17:22:10 1999 Page: 1

Inlet B:	On	270 C
Detector A:	Off	50 C
Detector B:	On	290 C
Auxiliary:	Off	50 C

Oven Parameters:

Oven Equib Time:	0.50 minutes
Oven Max:	300 C
Oven State:	On
Cryo State:	Off
Cryo Blast:	Off
Ambient:	25 C

Oven Program:

Initial Temperature:	120 C
Initial Time:	1.00 minutes

Level	Rate (C/minute)	Final Temperature (C)	Final Time (minutes)
1	8.0	200	0.00
2(A)	20.0	290	6.00
3(B)	0.0	0	0.00

Next Run Time: 21.50 minutes

HP5890 Purge Valve Settings

Inlet Purge	Init Value	On Time	Off Time	Splitless Injection
A	Off	1.00	0.00	No
B	Off	0.75	0.00	Yes

HP5890 Valve and Relay Information

Initial Setpoints:

5890 Valves:

Valve 1: Off Valve 2: Off Valve 3: Off Valve 4: Off

19405 Valves:

Valve 5: Off Valve 6: Off Valve 7: Off Valve 8: Off

19405 Relays:

Relay 1: Off Relay 2: Off Relay 3: Off Relay 4: Off

HP5890 Detector Information

Detector	Type	State
A	---	Off
B	---	Off

HP5890 Signal Information

Method: PSIM2.M Wed Jun 02 17:22:10 1999 Page: 2

Signal	Source	Peak Width	Data Rate	Start Data	Stop Data
1	Testplot	0.053	5.000	0.00	1.00
2	Testplot	0.053	5.000	0.00	1.00

MS ACQUISITION PARAMETERS

General Information
------- -----------

Tune File : high.u
Acquistion Mode : SIM

MS Information
-- -----------

Solvent Delay : 5.00 min

EM Absolute : False
EM Offset : 0
Resulting EM Voltage : 2223.5

[Sim Parameters]

GROUP 1
Group ID : 1
Resolution : Low
Group Start Time : 0.00
Plot 1 Ion : 165.0

Ions/Dwell In Group	(Mass, Dwell)	(Mass, Dwell)	(Mass, Dwell)
	(165.0, 70)	(164.0, 70)	(141.0, 70)
	(136.0, 70)	(94.0, 70)	(99.0, 70)

END OF MS ACQUISITION PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

DATA ANALYSIS PARAMETERS

Method Name: C:\HPCHEM\1\METHODS\PSIM2.M

Method: PSIM2.M Wed Jun 02 17:22:10 1999 Page: 3

Percent Report Settings

Sort By: Signal

Output Destination
    Screen: No
    Printer: Yes
    File: No

Integration Events: Meth Default

Generate Report During Run Method: No

Signal Correlation Window: 0.020

Qualitative Report Settings

Peak Location of Unknown: Apex

Library to Search	Minimum Quality
DEMO.L	0

Integration Events: Meth Default

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: No

Quantitative Report Settings

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: Yes

Reference Window: 2.00 Minutes
Non-Reference Window: 1.00 Minutes
Correlation Window: 0.10 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information

---

1) Acenaphthene-d10 (ISTD)

Ret. Time 7.00 min., Extract & Integrate from 6.50 to 7.50 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 164.00			*** METH DEFAULT ***
Q1 165.00	12.10	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		0.500	180648
2		0.500	163623
3		0.500	168789
4		0.500	161303
5		0.500	165643
6		0.500	158435

Qualifier Peak Analysis ON ISTD conc: 0.500 ppm
Curve Fit: Linear

---

2) Methamidophos ( )

Ret. Time 6.46 min., Extract & Integrate from 5.96 to 6.96 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 94.00			*** METH DEFAULT ***
Q1 141.00	30.40	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	222233
2		0.500	88636
3		0.250	43092
4		0.100	16666
5		0.025	4299
6		0.050	6883

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

3) Acephate ( )

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 136.00			*** METH DEFAULT ***
Q1 94.00	86.50	20.0	*** METH DEFAULT ***
Q2 141.00	1.40	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	82378
2		0.500	23384
3		0.250	10193
4		0.100	3650
5		0.025	1073
6		0.050	1803

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

4) Tributylphosphate ( )

Ret. Time 10.89 min., Extract & Integrate from 10.39 to 11.39 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 99.00			*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	826082
2		0.500	307882
3		0.250	134318
4		0.100	46245
5		0.025	11617
6		0.050	22558

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

END OF DATA ANALYSIS PARAMETERS

Appendix B

Method PSIM.M from Chemstation

Method Information For: C:\HPCHEM\1\METHODS\PSIM.M

Method Sections To Run:

( ) Save Copy of Method With Data
( ) Pre-Run Cmd/Macro =
(X) Data Acquisition
(X) Data Analysis
( ) Post-Run Cmd/Macro =

Method Comments:
This is the SIM method for Azinphos methyl, Chlorpyrifos, Daizinon, Malathion and their oxons

END OF TOPLEVEL PARAMETERS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet: GC
Injection Source: GC ALS
Mass Spectrometer: Enabled

HP GC Injector

Front Injector:
No parameters specified

Back Injector:

Sample Washes	1	
Sample Pumps	4	
Injection Volume	2.0	microliters
Syringe Size	10.0	microliters
On Column	Off	
Nanoliter Adapter	Off	
PostInj Solvent A Washes	3	
PostInj Solvent B Washes	3	
Viscosity Delay	0	seconds
Plunger Speed	Fast	

HP5890 Temperature Parameters

Inlet A:	Off	50 C
Inlet B:	On	270 C
Detector A:	Off	50 C
Detector B:	On	290 C
Auxiliary:	Off	50 C

Oven Parameters:

Oven Equib Time:	0.50 minutes
Oven Max:	300 C
Oven State:	On
Cryo State:	Off
Cryo Blast:	Off
Ambient:	25 C

Oven Program:

Initial Temperature:	150 C
Initial Time:	2.00 minutes

Level	Rate (C/minute)	Final Temperature (C)	Final Time (minutes)
1	8.0	290	6.00
2(A)	0.0	50	1.00
3(B)	0.0	50	1.00
Next Run Time:		25.50 minutes	

HP5890 Purge Valve Settings

Inlet Purge	Init Value	On Time	Off Time	Splitless Injection
A	Off	1.00	0.00	No
B	Off	0.75	0.00	Yes

HP5890 Valve and Relay Information

Initial Setpoints:

5890 Valves:				
	Valve 1: Off	Valve 2: Off	Valve 3: Off	Valve 4: Off
19405 Valves:				
	Valve 5: Off	Valve 6: Off	Valve 7: Off	Valve 8: Off
19405 Relays:				
	Relay 1: Off	Relay 2: Off	Relay 3: Off	Relay 4: Off

HP5890 Detector Information

Detector	Type	State
A	---	Off
B	---	Off

Method: PSIM.M Wed Jun 02 17:22:27 1999 Page: 2

Not saving signal data.

Signal	Source	Peak Width	Data Rate	Start Data	Stop Data
1	Testplot	0.053	5.000	0.00	1.00
2	Testplot	0.053	5.000	0.00	1.00

MS ACQUISITION PARAMETERS

General Information
------- -----------

Tune File : high.u
Acquistion Mode : SIM

MS Information
-- -----------

Solvent Delay : 5.00 min

EM Absolute : False
EM Offset : 47
Resulting EM Voltage : 2270.6

[Sim Parameters]

GROUP 1
Group ID : 1
Resolution : Low
Group Start Time : 0.00
Plot 1 Ion : 137.0
Ions/Dwell In Group

(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)
(137.0,	70)	(188.0,	70)	(153.0,	70)
(179.0,	70)	(273.0,	70)	(298.0,	70)

GROUP 2
Group ID : 2
Resolution : Low
Group Start Time : 11.00
Plot 1 Ion : 127.0
Ions/Dwell In Group

(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)
(127.0,	70)	(125.0,	70)	(173.0,	70)
(195.0,	70)	(197.0,	70)	(199.0,	70)
(298.0,	70)	(314.0,	70)		

GROUP 3
Group ID : 3
Resolution : Low
Group Start Time : 16.00
Plot 1 Ion : 132.0
Ions/Dwell In Group

(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)
(132.0,	100)	(160.0,	70)	(326.0,	70)

Method: PSIM.M Wed Jun 02 17:22:27 1999 Page: 3

END OF MS ACQUISITION PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

DATA ANALYSIS PARAMETERS

Method Name: C:\HPCHEM\1\METHODS\PSIM.M

Percent Report Settings

Sort By: Signal

Output Destination
Screen: No
Printer: Yes
File: No

Integration Events: Meth Default

Generate Report During Run Method: No

Signal Correlation Window: 0.020

Qualitative Report Settings

Peak Location of Unknown: Apex

Library to Search	Minimum Quality
DEMO.L	0

Integration Events: Meth Default

Report Type: Summary

Output Destination
Screen: No
Printer: Yes

Generate Report During Run Method: No

Quantitative Report Settings

Report Type: Summary

Output Destination
Screen: No
Printer: Yes
File: No

Generate Report During Run Method: Yes

Organophshorus Pesticide Analysis
Calibration Last Updated: Sun May 30 09:23:25 1999

Reference Window: 1.00 Minutes
Non-Reference Window: 0.50 Minutes
Correlation Window: 0.10 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information

---

1) Phenanthrene-d10 (ISTD)

Ret. Time 9.00 min., Extract & Integrate from 8.50 to 9.50 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 188.00			*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		0.500	339278
6		0.500	349264
3		0.500	322776
4		0.500	359274
5		0.500	303037
2		0.500	435237

Qualifier Peak Analysis ON ISTD conc: 0.500 ppm
Curve Fit: Linear, forced through origin

---

2) Diazinon ( )

Ret. Time 8.54 min., Extract & Integrate from 8.04 to 9.04 min.

Method: PSIM.M Wed Jun 02 17:22:27 1999 Page: 5

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	179.00			*** METH DEFAULT ***
Q1	137.00	143.70	20.0	*** METH DEFAULT ***
Q2	153.00	48.80	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	103212
6		0.025	2853
3		0.250	23416
4		0.100	10249
5		0.050	4795
2		0.500	65844

Qualifier Peak Analysis ON
Curve Fit: Linear, forced through origin

---

3) Diazinon O analog ( )

Ret. Time 9.86 min., Extract & Integrate from 9.36 to 10.36 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	273.00			*** METH DEFAULT ***
Q1	288.00	0.00	20.0	*** METH DEFAULT ***
Q2	137.00	145.80	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	136236
6		0.025	3598
3		0.250	30377
4		0.100	13648
5		0.050	5950
2		0.500	86451

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

4) Chlorpyrifos ( )

Ret. Time 11.15 min., Extract & Integrate from 10.65 to 11.65 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	197.00			*** METH DEFAULT ***
Q1	199.00	92.90	20.0	*** METH DEFAULT ***
Q2	214.00	0.00	20.0	*** METH DEFAULT ***
Q3	125.00	76.00	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	115295
6		0.025	3610
3		0.250	25780
4		0.100	11836
5		0.050	7122
2		0.500	68437

5) Malathion ( )

?et. Time 12.36 min., Extract & Integrate from 11.86 to 12.86 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	173.00			*** METH DEFAULT ***
Q1	125.00	136.80	20.0	*** METH DEFAULT ***
Q2	127.00	103.10	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	145825
6		0.025	3011
3		0.250	30572
4		0.100	12990
5		0.050	5202
2		0.500	91458

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

6) Malathion O analog ( )

Ret. Time 12.73 min., Extract & Integrate from 12.23 to 13.23 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	195.00			*** METH DEFAULT ***
Q1	173.00	72.00	20.0	*** METH DEFAULT ***
Q2	127.00	797.40	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	31345
6		0.025	916
3		0.250	7122
4		0.100	3266
5		0.050	1366
2		0.500	19787

Qualifier Peak Analysis ON
Curve Fit: Quadratic

7) Chlorpyrifos O analog ( )

Ret. Time 12.91 min., Extract & Integrate from 12.41 to 13.41 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	199.00			*** METH DEFAULT ***
Q1	197.00	118.50	20.0	*** METH DEFAULT ***
Q2	298.00	56.10	20.0	*** METH DEFAULT ***
Q3	173.00	7.70	20.0	*** METH DEFAULT ***

Method: PSIM.M Wed Jun 02 17:22:27 1999 Page: 7

Lvl	ID	Conc (ppm)	Response
		1.000	59727
6		0.025	1711
3		0.250	14117
4		0.100	6448
5		0.050	3720
2		0.500	37556

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

8) Chrysene-d12 (ISTD)

Ret. Time 16.57 min., Extract & Integrate from 16.07 to 17.07 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 240.00			*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		0.500	218692
6		0.500	231760
3		0.500	213049
4		0.500	273102
5		0.500	190411
2		0.500	284433

Qualifier Peak Analysis ON ISTD conc: 0.500 ppm
Curve Fit: Linear, forced through origin

---

9) Triphenylphosphate ( )

Ret. Time 16.27 min., Extract & Integrate from 15.77 to 16.77 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 326.00			*** METH DEFAULT ***
Q1 77.00	136.80	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	153029
6		0.025	8182
3		0.250	36864
4		0.100	19258
5		0.050	8423
2		0.500	98576

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

10) Guthion ( )

Ret. Time 18.52 min., Extract & Integrate from 18.02 to 19.02 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 160.00			*** METH DEFAULT ***
Q1 132.00	93.20	20.0	*** METH DEFAULT ***
Q2 77.00	179.80	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	98475
6		0.025	999
3		0.250	18398
4		0.100	8706
5		0.050	2718
2		0.500	60445

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

11) Guthion O analog ( )

Ret. Time 18.81 min., Extract & Integrate from 18.31 to 19.31 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 160.00			*** METH DEFAULT ***
Q1 132.00	106.70	20.0	*** METH DEFAULT ***
Q2 77.00	169.60	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	84221
6		0.025	62
3		0.250	13102
4		0.100	5471
5		0.050	607
2		0.500	54436

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

END OF DATA ANALYSIS PARAMETERS

Appendix C

Chromatograms

Data File : C:\HPCHEM\1\DATA\OPC666.D Vial: 1
Acq On : 30 May 1999 1:54 pm Operator:
Sample : 1.00 ppm opc std 070130 Inst : GC/MS Ins
Misc : Multiplr: 1.00
MS Integration Params: rteint.p
Quant Time: May 30 14:20 1999 Quant Results File: PSIM.RES

Quant Method : C:\HPCHEM\1\METHODS\PSIM.M (RTE Integrator)
Title : Organophshorus Pesticide Analysis
Last Update : Sun May 30 09:23:25 1999
Response via : Initial Calibration
DataAcq Meth : PSIM

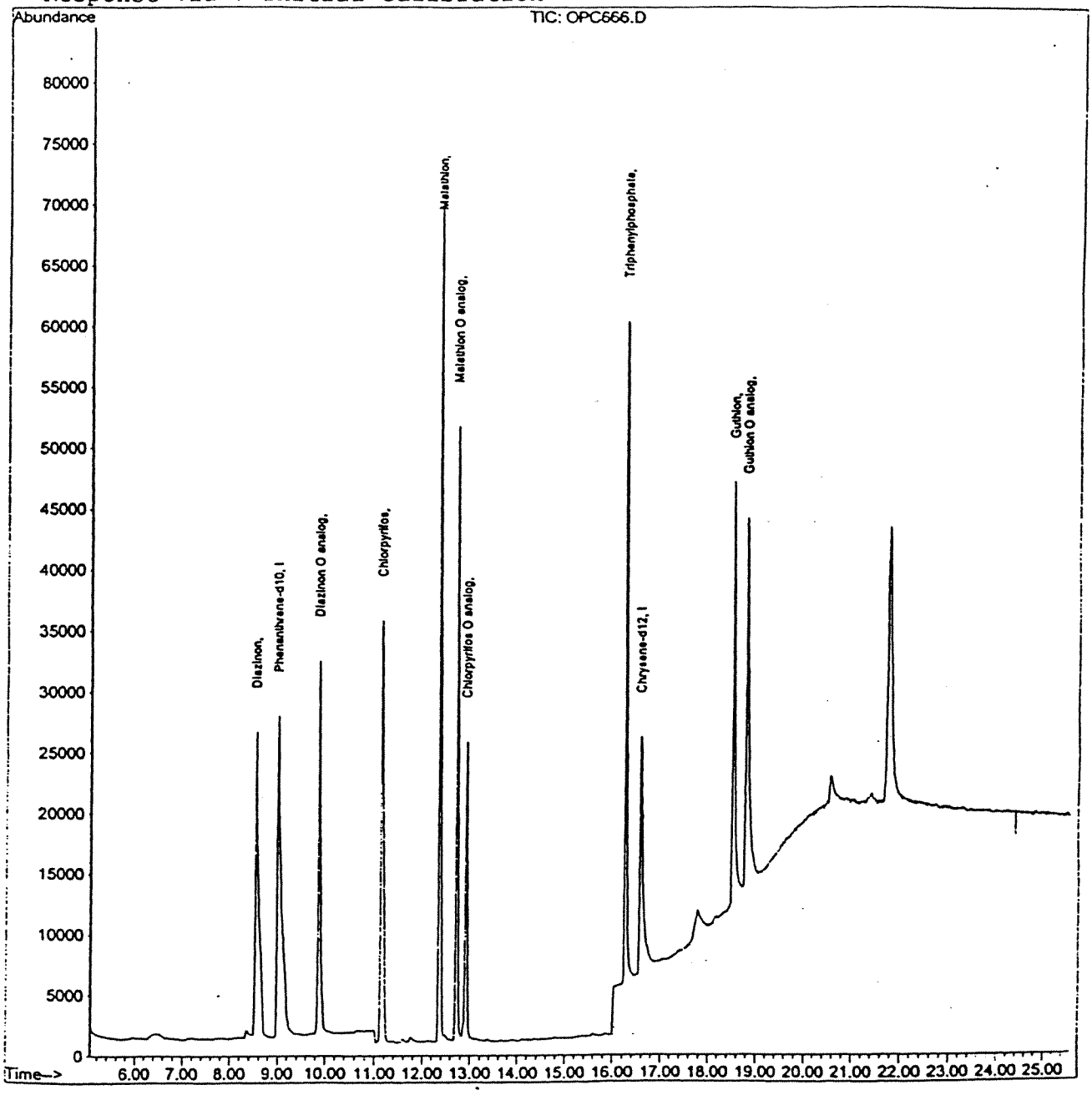
Internal Standards	R.T.	QIon	Response	Conc	Units		Dev(Min)
1) Phenanthrene-d10	9.00	188	156103	0.50	ppm		0.00
8) Chrysene-d12	16.56	240	94420	0.50	ppm		0.00
System Monitoring Compounds							
Target Compounds							Qvalue
2) Diazinon	8.54	179	42212	1.01	ppm		93
3) Diazinon O analog	9.86	273	39878	0.99	ppm	#	95
4) Chlorpyrifos	11.15	197	51224	1.00	ppm	#	74
5) Malathion	12.36	173	53711	1.00	ppm		97
6) Malathion O analog	12.72	195	10535	1.00	ppm		89
7) Chlorpyrifos O analog	12.91	199	22333	1.01	ppm		93
9) Triphenylphosphate	16.26	326	60643	1.01	ppm		98
10) Guthion	18.51	160	35740	1.00	ppm		90
11) Guthion O analog	18.79	160	39813	1.01	ppm		87

(#) = qualifier out of range (m) = manual integration
OPC666.D PSIM2.M Wed Jun 02 17:46:26 1999 RPT1 Page 1

Data File : C:\HPCHEM\1\DATA\OPC666.D
Acq On : 30 May 1999 1:54 pm
Sample : 1.00 ppm opc std 070130
Misc :
MS Integration Params: rteint.p
Quant Time: May 30 14:20 1999

Vial: 1
Operator:
Inst : GC/MS Ins
Multiplr: 1.00
Quant Results File: PSIM.RES

Method : C:\HPCHEM\1\METHODS\PSIM2.M (RTE Integrator)
Title : Acephate and Methamidophos Analysis
Last Update : Fri May 07 19:22:17 1999
Response via : Initial Calibration



OPC666.D PSIM2.M Wed Jun 02 17:46:28 1999 RPT1 Page 2

Data File : C:\HPCHEM\1\DATA\OPC491.D Vial: 1
Acq On : 18 May 1999 8:56 pm Operator:
Sample : 1.0 ppm Acephate and Methamidophos Inst : GC/MS Ins
Misc : Multiplr: 1.00
MS Integration Params: rteint.p
Quant Time: May 18 21:18 1999 Quant Results File: PSIM2.RES

Quant Method : C:\HPCHEM\1\METHODS\PSIM2.M (RTE Integrator)
Title : Acephate and Methamidophos Analysis
Last Update : Fri May 07 19:22:17 1999
Response via : Initial Calibration
DataAcq Meth : PSIM2

Internal Standards	R.T.	QIon	Response	Conc	Units		Dev(Min)
1) Acenaphthene-d10	7.26	164	152719	0.50	ppm		0.18
System Monitoring Compounds							
Target Compounds							Qvalue
2) Methamidophos	6.67	94	54803	0.92	ppm		97
3) Acephate	10.31	136	19326	0.77	ppm	#	94
4) Tributylphosphate	11.13	99	258525	1.06	ppm		100

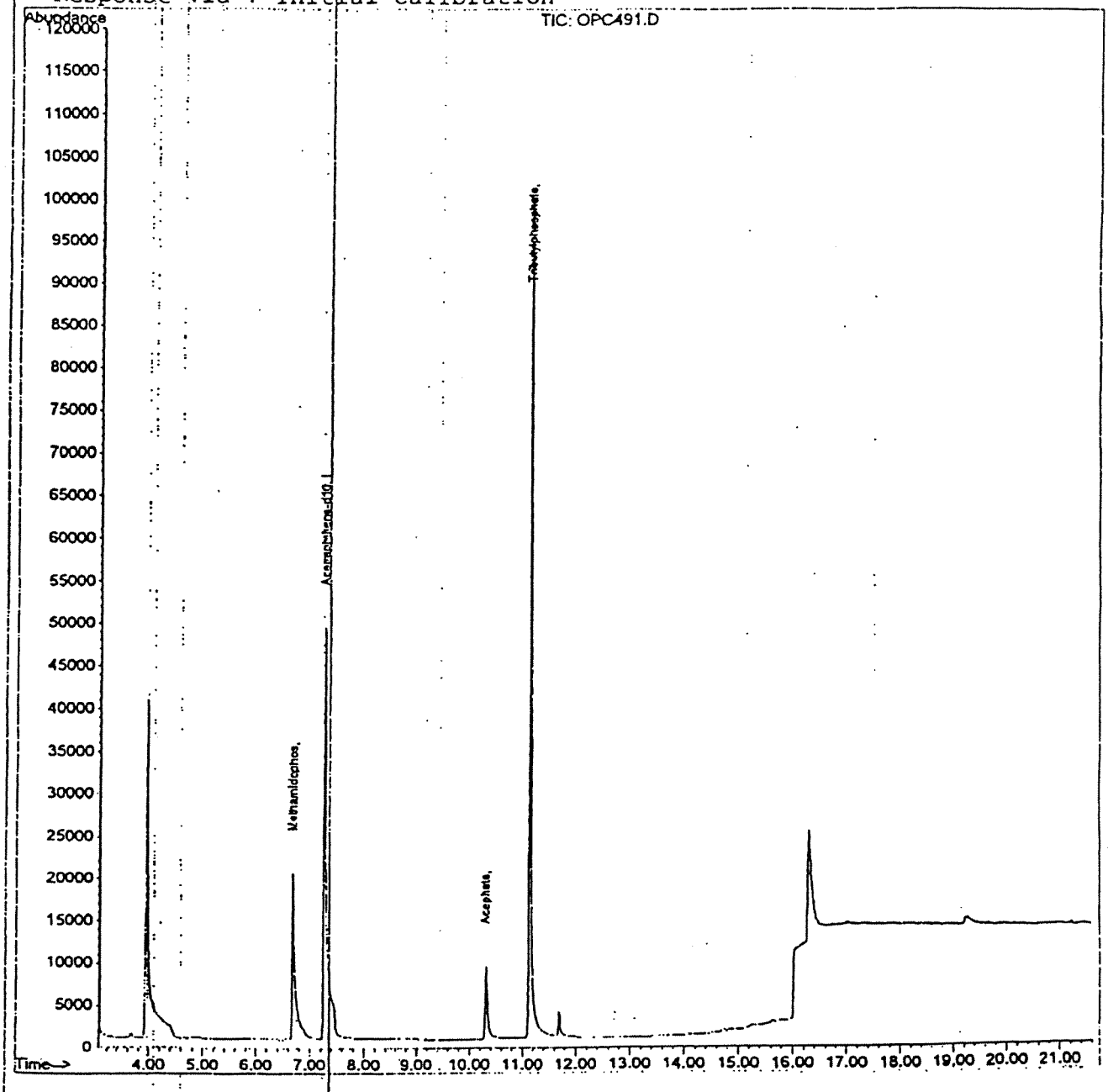
(#) = qualifier out of range (m) = manual integration
OPC491.D PSIM2.M Wed Jun 02 17:47:23 1999 RPT1 Page 1

Data File : C:\HPCHEM\1\DATA\OPC491.D
Acq On : 18 May 1999 8:56 pm
Sample : 1.0 ppm Acephate and Methamidophos
Misc :
MS Integration Params: rteint.p
Quant Time: May 18 21:18 1999

Vial: 1
Operator:
Inst : GC/MS Ins
Multiplr: 1.00

Quant Results File: PSIM2.RES

Method : C:\HPCHEM\1\METHODS\PSIM2.M (RTE Integrator)
Title : Acephate and Methamidophos Analysis
Last Update : Fri May 07 19:22:17 1999
Response via : Initial Calibration



OPC491.D PSIM2.M Wed Jun 02 17:47:32 1999 RPT1 Page 2

ORGANOPHOSPHORUS PESTICIDES

Compound	Retention Time (minutes)	Quantitation Ion (m/z)	Confirmation Ion 1 (m/z)	Confirmation Ion 2 (m/z)
Acephate	7.58	136	94	137
Azinphos methyl	20.84	160	132	none
Azinphos methyl oxon	20.14	160	132	none
Chlorpyrifos	14.51	197	199	314
Chlorpyrifos oxon	15.05	197	199	298
Diazinon	10.86	137	179	153
Diazinon oxon	11.31	137	273	288
Malathion	15.01	125	127	173
Malathion oxon	14.60	127	195	173
Methamidophos	3.92	94	141	136
Tributylphosphate	7.98	99	none	none
Triphenylphosphate	18.37	326	186	none
Acenaphthene-d_{10}	5.00	164	162	none
Phenanthrene-d_{10}	10.82	188	none	none
Chrysene-d_{12}	18.89	240	none	none

PROTOCOL AMENDMENT FORM

COPY

AMENDMENT NUMBER: 1

Protocol: En-fate Study No. 00102

Protocol Title: COMMUNITY WATER SYSTEM SURFACE DRINKING WATER MONITORING STUDY FOR ORGANOPHOSPHATE PESTICIDES AND THEIR MAJOR DEGRADATION PRODUCTS IN THE UNITED STATES

Compound/Formulation: Acephate, Azinphos-methyl, Chlorpyrifos, Diazinon, Malathion, Methamidophos and major degradation products.

AMENDMENT(S):

1) SECTION 6: ANALYTICAL METHODOLOGY

CHANGES: Analytical methodologies for the above referenced compounds as per SOP NO.: EASI MS-20.00.

REASON(S): Analytical methodologies were modified to reflect the changes incorporated to increase the recovery of the azinphos-methyl oxygen analog. Extension of the analysis time period from thirty days after extraction to forty days after extraction.

2) Proposed Experimental Termination Date

CHANGES: Proposed completion date extended to September 30, 1999.

REASON(S): Analytical recovery for the azinphos-methyl oxygen analog was not within method specifications. Method development for the azinphos-methyl oxygen analog took additional time and delayed the analysis of samples and the preparation of the report.

Effect of change: Delays reporting of the data until September 30, 1999.

Effective date of this Amendment: August 12, 1999

STUDY DIRECTOR: _____ DATE 8-16-99

Amendments to be distributed per Protocol Distribution List

ORGANOPHOSPHORUS PESTICIDES

References: USGS Open-File Report 95-181 "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Determination of Pesticides in Water by C-18 Solid-Phase Extraction and Capillary-Column Gas Chromatography/Mass Spectrometry with Selected-Ion Monitoring", 1995

US EPA Test Methods for Evaluating Solid Waste, SW-846, 3rd edition, Method 8141A.

US EPA 40 CFR Part 136, Appendix B. "Definition and Procedure for the Determination of the Method Detection Limit"

US EPA Method 1618: Organo-halide Pesticides, Organo-phosphorus Pesticides, and Phenoxy-acid Herbicides by Wide Bore Capillary column Gas Chromatography with selective Detectors. July 1989.

Holding Time: All samples must be extracted within 7 days of collection and sample extracts should be analyzed within 40 days of extraction. Disposal of samples will be only with the approval of the study director.

Preservation: Sample container must contain sodium thiosulfate at approximately 0.01% to quench the redox potential of any residual chlorine or chloramine that may be added by a community water system. All samples must be protected from light and refrigerated at 4°C ± 2°C from the time of collection until extraction.

Sampling: For water samples, 1 L of water is required for extraction and should be collected in sufficient volume for a second analysis, i.e. ≥ 2 liters. Samples must be collected in amber glass containers.

1.0 Scope and application

This method covers the determination of several organophosphorus pesticides and their degradates. This SOP covers sample preparation and analysis. The analytical method was designed to analyze water samples for the presence of organophosphorus pesticides and their primary degradates.

The following compounds (target analytes) are determined by this method:

COMPOUND	CAS No.[a]	MDL(ppb)[b]	PQL(ppb)[b]
Acephate	30560-19-1	0.0320	0.058
Azinphos Methyl	86-50-0	0.0100	0.050
Azinphos methyl oxon	961-22-8	0.0131	0.065
Chlorpyrifos	2921-88-2	0.0089	0.044
Chlorpyrifos oxon	5598-15-2	0.0070	0.035
Diazinon	333-41-5	0.0058	0.029
Diazinon oxon	962-58-3	0.0088	0.044
Malathion	121-75-5	0.0086	0.043
Malathion oxon	1634-78-2	0.0085	0.042
Methamidophos	10265-92-6	0.0170	0.039

[a]: Chemical Abstracts Service Number
[b]: Method Detection Limit and Practical Quantitation Limit as determined by the laboratory upon spiking drinking water from a local treatment facility.

Detection limits of this method are dependent upon the levels of interferences and instrumental limitations. The limits in the table above typify the minimum quantities that can be detected in water treatment facility effluents. The practical quantitation limit (PQL) is generally accepted as 5 times the MDL. The MDL is determined by multiplying the standard deviation of ≥ 7 analyses by the student t value appropriate for that number of analyses (n-1) at the 99% confidence level.

2.0 Summary of Method

Solid phase extraction procedures are employed for aqueous samples. Analysis is accomplished by injection of a fixed volume of an extract onto a gas chromatographic column equipped with a fused silica capillary column and detection using a mass selective detector in the selected ion mode.

3.0 Interferences

Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of analysis by running laboratory reagent blanks.

All reagents are to be tested prior to use to ensure that interferences do not affect analyses.

4.0 Apparatus and Materials

Gas Chromatograph - Hewlett Packard 5890 Series II.

Autosampler, autoinjector - Hewlett Packard 7673.

Mass spectrometer data system

Mass Selective Detector HP 5971A operated in selected ion mode.

Column
Restek-Rtx-200, 15 m length x 0.25 mm inner diameter x 1.0 µm film thickness Restek # 15050 or equivalent

65 mm glass powder funnels

Nitrogen evaporation device (laboratory constructed)

Rotary evaporator Bucchi Model # R-3000 or equivalent

Autosampler vials with teflon-lined crimp top seals

Vacuum manifold for eluting multiple C-18 extraction disks - Baker Speedisk 47 mm Baker # 8095-06 or equivalent with Gast vacuum pump or equivalent

Vacuum or peristaltic pump manifold for eluting multiple cartridges - Baker # 7018-00 or equivalent with Gast vacuum pump or equivalent

Vacuum extraction apparatus for eluting multiple sorbent cartridges (laboratory constructed) with Masterflex peristaltic pump or equivalent

Baker Speedisk C18 SPE disks Baker #8055-06 or equivalent

Waters Sep-Pak Plus AC-2 cartridges Waters Custom # WAT020585 (ref#JJAN20229) or equivalent

SPE Polyethylene Reservoirs, 75 ml Baker # 7120-03 or equivalent

SPE Polyethylene Reservoirs, 15 ml Baker with snap tops and 1.5' of 1/16" i.d., 1/8" o.d. teflon tubing attached to snap tops

4.0 Apparatus and Materials, cont.

Pyrex glass wool

pH paper - range of 1-12

Pasteur pipettes, disposable borosilicate glass - 5.75" and 9"

Microsyringes - 10 µl, 25 µl, 50 µl, 100 µl, 250 µl, 500 µl, 5 ml and 10 ml, Hamilton models or equivalent

Class A Graduated cylinders – 1000 ml and 250 ml capacity

Class A Volumetric flasks – 10.0 ml, 25.0 ml, 50.0 ml, and 100 ml

40 ml precleaned vials

15 ml graduated conical centrifuge tubes

250 ml round bottom flasks

50 ml pear shaped evaporation flasks with 24/40 joint

Analytical balance - capable of accurately weighing 10 g ± 0.0001g, Denver Instruments Model A-250 or equivalent

Small stainless steel spatulas

5.0 Reagents and Standards

Chemicals and Reagents

Acephate - Valent U.S.A. Corporation Lot # AS 40p or equivalent

Azinphos Methyl (Guthion) - Bayer Corporation Lot # K-791 or equivalent

Azinphos Methyl oxon - Bayer Corporation Lot # K-166 or equivalent

Chlorpyrifos - Dow AgroSciences Lot # MM 939593-17 or equivalent

Chlorpyrifos oxon - Dow AgroSciences Lot # GS-33-82:126 or equivalent

Diazinon - Novartis Crop Protection Lot # S97-2127 or equivalent

Diazinon oxon - Novartis Crop Protection Lot # S97-2011or equivalent

Malathion - Cheminova Agro A/S Lot # 324-OSJ-54C or equivalent

Malathion oxon - Cheminova Agro A/S Lot # 270-ABB-09-01 or equivalent

Methamidophos - Bayer Corporation Lot # K-753 or equivalent

Triphenylphosphate - Chem Service # O-921 or equivalent

Tributylphosphate - Chem Service # F2191 or equivalent

Semivolatile GCMS Internal Standard Mix, 2000 ng/ul - Ultra Scientific # ISM-560 or equivalent

Perfluorotributylamine (PFTBA)

Organic free water - carbon filtered, deionized water

Laboratory potable water (for matrix spikes where applicable)

Hydrochloric acid 1N – Prepared by adding 80 ml of conc. HCl to 880 ml of deionized water

Acetone - ACS reagent grade

5.0 Reagents and Standards, cont.

Methanol - ACS reagent grade

Methylene Chloride - ACS reagent grade

Ethyl Acetate - ACS reagent grade

Sodium sulfate – granular, anhydrous, ACS reagent grade. Each lot must be extracted with 1:1 methylene chloride:ethyl acetate and analyzed by GC/MS/SIM to demonstrate that it is free of interference before use. Caution: An open container of sodium sulfate may become contaminated during storage in the laboratory.

Helium carrier gas, ultrapure

Nitrogen gas

Stock standard solutions

The preparation of all standards will be documented in the organics standards logbook. Each entry is to be signed and dated by the analyst. The entry should contain adequate information as to how the standard was prepared and how it should be used. The standards should be labeled using the number of the standard logbook and applicable page number to facilitate traceability as well as a short description of the standard, the concentration and the expiration date. Due to the small vials used for some calibration standards, the concentration may be eliminated from the label if the standard concentration is known by the label as a working standard and the concentration is traceable to the logbook using the standard I.D.

Stock standards shall be prepared from analytical standards supplied by and characterized in accordance with FIFRA GLPs by the sponsor. It is the responsibility of the sponsor to maintain adequate documentation that verifies compound purity, concentration and identity. Any test/control/reference substances used in the study must be characterized prior to its use in the study. The laboratory will maintain copies of sponsor GLP-certification information in the neat standards logbook.

The surrogate compounds and internal standard compounds are not characterized in accordance with FIFRA GLPs.

If the standards are prepared in the lab care must be exercised to prevent contamination. Glassware must be scrupulously clean. Use high quality solvents and reference materials assayed at 97% or greater purity.

Calibrate the balance according to the balance SOP. Prepare stock standards by accurately weighing the neat compound to the nearest 0.0001g. Place the volumetric flask to be used on the balance, tare the balance and quantitatively transfer the compound to the flask using a small stainless steel spatula. The mass of compound to be weighed is dependent upon the amount of standard available and the size of the volumetric dilution flask. When possible, use a 10.0 ml volumetric flask for preparation of stock standards. When possible, i.e. when the quantity of neat standard is sufficient, prepare standards to provide a final concentration of approximately 10,000 ug/ml. For liquid neat standards, use an appropriate microsyringe for optimal control of standard addition during weighing and quantitatively transfer to the tared volumetric flask. Dilute stock standards to volume with ACS-grade acetone. Stocks may be prepared as single components or as mixtures.

Store stock standards in glass screw top vials with Teflon septa. Store at <0°C. Standards may be stored up to 1 year unless the standards show signs of degradation.

Prepare working standard solutions from stock or intermediate standards for direct analysis on the GC/MS as follows:

Diazinon, Chlorpyrifos, Malathion, Guthion, and their Oxons

Prepare 1.0 ml each of 6 working standards by diluting (adding) the appropriate amounts of the stock standard solutions (depending on the exact concentration of the stock or intermediate standard) to give the following concentrations: 0.025, 0.050, 0.100, 0.250, 0.500, and 1.00 µg/ml. The 0.025 µg/ml standard is included in the run sequence and analyzed for verification of instrument sensitivity. All working standards for the above compounds are prepared in solvent previously passed through C-18 extraction media and prepared exactly as described for deionized method blanks.

Acephate and Methamidophos

Prepare 1.0 ml each of 6 working standards by diluting (adding) the appropriate amounts of the stock standard solutions in acetone (depending on the exact concentration of the stock or intermediate standard) to give the following concentrations: 0.0125, 0.025, 0.050, 0.100, 0.250, and 0.500 µg/ml. The 0.0125 µg/ml standard is included in the run sequence and analyzed for verification of instrument sensitivity.

All working standards are prepared in 1.0 ml crimp top ALS vials and must be stored at <0°C. Working standard solutions must be crimped immediately after use and may be stored up to one month unless standards show signs of degradation.

Internal Standards

Begin with a 2000 ng/µl semivolatile GCMS internal standard solution (Ultra Scientific #ISM-560, 2000 ng/ul or equivalent). Prepare a 50 ng/µl working solution by diluting 0.25 ml (250 µl of the stock to 10.0 ml with ethyl acetate. Each 1.0 ml of sample extract should be spiked with 10.0 µl of internal standard solution immediately prior to analysis. The internal standard compounds used are acenaphthene-d_{10}, phenanthrene-d_{10}, and chrysene-d_{12}.

Surrogate Standards

The surrogates used are tributylphosphate and triphenylphosphate. The surrogate solution is spiked prior to extraction using a 0.20 ug/ml working solution (prepared as described below).

C-18 Extraction: Diazinon, Chlorpyrifos, Malathion, Acephate and their Oxons
Prepare a stock solution by weighing 0.100g of triphenylphosphate and dissolving in methylene chloride and bringing to volume in a 10.0 ml volumetric flask. Prepare a 50 µg/ml standard solution by diluting 0.050 ml (50 µl) of this stock solution to 10.0 ml in a volumetric flask with acetone. Prepare a 0.20 µg/ml surrogate working solution by diluting 0.10 ml (100 µl) of the 50 µg/ml solution to 25.0 ml in a volumetric flask with acetone. Spike 1.0 ml of a 0.20 µg/ml surrogate solution in acetone into each 1000 ml aliquot of sample to be extracted.

AC-2 Extraction: Acephate and Methamidophos
Prepare the stock solution and 50 µg/ml standard solution exactly as described above for the C-18 extraction using tributylphoshate. Prepare the final 0.20 ug/ml surrogate working solution as described above replacing acetone with deionized water as the solvent. Spike 0.25 ml (250 µl) into each 250 ml aliquot of sample to be extracted.

Note: Blank and matrix spikes are not spiked separately with the surrogates. The surrogate is prepared with the matrix spiking solution and is added when the sample is spiked with the matrix spike working solution.

Pesticide Matrix Spike Solution

Prepare a matrix spiking solution containing the surrogate compounds as follows.

C-18 Extraction: Diazinon, Chlorpyrifos, Malathion, Acephate and their Oxons
Prepare a stock solution by weighing 0.100g of each neat compound and the surrogate triphenylphosphate and dissolving in methylene chloride and bringing to volume in a 10.0 ml volumetric flask. Prepare a 50 µg/ml intermediate standard solution by diluting 0.050 ml (50 µl) of this stock solution to 10.0 ml in a volumetric flask with acetone. Prepare a 0.20 µg/ml working solution by diluting 0.10 ml (100 µl) of the 50 µg/ml solution to 25.0 ml in a volumetric flask with acetone. Spike 1.0 ml of the 0.20 µg/ml surrogate solution in acetone into each 1000 ml aliquot of sample to be extracted.

AC-2 Extraction: Acephate and Methamidophos

Prepare acephate and methamidophos stock, intermediate, and working standards exactly as described above for the C-18 extraction with the following exception: use tributylphosphate as the surrogate. Spike 0.25 ml of the 0.20 µg/ml surrogate solution into an empty 250 ml round-bottom flask and allow the acetone to evaporate by placing the flask under a vacuum hood and air drying with the hood on pulling the sash down before adding samples.

Store all standards and matrix spiking solutions at <0°C.

6.0 Procedures

Calibration of equipment

Mass spectrometer performance evaluation

Tune the mass spectrometer daily using the procedure and software provided by the manufacturer. Parameters in tuning are set to give ± 0.15 atomic mass unit resolution at masses 69, 219, and 414 in the spectrum of perfluorotributylamine. Adjust the electron multiplier to get a minimum area of 1,000,000 counts for mass 69 ion. Manually adjust, if necessary, so that the mass 69 ion has 100 percent abundance, mass 219 ion is 40±20 percent, and mass 414 ion is 6.2 ±5.7 percent relative abundance. Check the mass assignments to ensure accuracy to ± 0.15 atomic mass unit in the spectrum scan and that mass peak widths measured at one-half the peak height range from 0.45 to 0.59 atomic mass unit in the profile report. Generate a tune report.

Initial calibration

Prepare an initial five point calibration by analyzing 2 µl each of the working standard concentrations specified in Section 5.0. Calibrate according to the same conditions prescribed in Appendices A and B, depending on the specific pesticides to be analyzed. Construct calibration curves using first order or quadratic fit using the five standards for each analyte. Select the fit, which introduces the least calibration error into the quantitation for each compound.

Tabulate the peak areas against concentration for each compound, surrogate, and internal standard.

The internal standard compounds used are acenaphthene-d_{10}, phenanthrene-d_{10}, and chrysene-d_{12} due to their similar chromatographic behavior to the compounds of interest. Note: Three additional internal standards are included in the internal mixture used for spiking but are not used for quantitation.

Calculate the response factor (RF_s) for each compound using the following equation:

$$\frac{A_s * C_{is}}{C_s * A_{is}} = RF_s$$

A_s = area of the sample peak
C_{is} = concentration of the internal standard
A_{is} = area of the internal standard
C_s = concentration of the standard compound

Initial calibration data are acceptable if the correlation coefficient, r, is ≥0.990 for linear and the coefficient of the determination, COD, is ≥ 0.990 for non-linear curves calculated across the working concentration range for each compound.

Continuing calibration

Prior to the analysis of each sample set at the beginning of each run sequence and every 10 samples thereafter during a series of analyses, analyze and evaluate a midpoint calibration solution containing all the selected compounds to ensure that the GC/MS performance is in compliance with all established criteria. Alternatively, a new five point calibration may be analyzed at the beginning of each run sequence.

Calculate the response factor of each compound in each subsequent standard analysis.

If the response for any analyte varies from the predicted response by more than ± 20 %, a new calibration curve must be prepared for that analyte or the data must be validated and qualified with a report narrative.

Sample Preparation

Extraction Procedure 1: For azinphos methyl, azinphos methyl oxon, chlorpyrifos, chlorpyrifos oxon, diazinon, diazinon oxon, malathion, malathion oxon

Remove samples from refrigerator and allow to reach ambient temperature.

Measure 1000 ml of sample into a graduated cylinder. Label each cylinder with the appropriate sample ID. Check pH with pH paper. Record the sample volume and pH in the extraction logbook.

Prepare a method blank and a matrix blank with each group of samples extracted. A method blank consists of a 1000 ml volume of laboratory deionized water. A matrix blank consists of a 1000 ml volume of laboratory potable water. Add approximately 0.1 g of sodium thiosulfate using a small stainless steel scoop and stir until dissolved.

For each sample selected for matrix spike and matrix spike duplicate analyses, measure out two additional 1000 ml aliquots.

Assemble the filter apparatus (EM-01) using Baker Speedisks C-18 SPE disks (or equivalent).

Preclean the extraction apparatus and disk by adding 5-10 ml of methylene chloride. Pull a small amount through the disk with vacuum; turn off the vacuum and allow the disk to soak for about two minutes. Pull the remaining solvent through the disk and allow disk to dry. Note: The vacuum apparatus is set to provide a maximum vacuum pressure between 20-25 mm Hg as measured by the inline pressure gauge. Do not adjust the vacuum pump to provide a vacuum greater than 25 mm Hg.

Repeat precleaning step.

Condition the disk by adding about 5-10 ml of methanol to the reservoir, pulling a small amount through the disk then letting it soak for about one minute. Pull most of the remaining methanol through the disk, leaving a visual layer of methanol above the surface of the disk. DO NOT ALLOW THE DISK TO GO DRY AT THIS POINT!

Add 5-10 ml of deionized water to the disk and pull through the disk leaving 3-5 mm (as measured by visual observation) of water above the surface of the disk.

Add 1.0 ml of surrogate spiking solution to the samples and 1.0 ml of matrix spiking solution to the designated matrix spike samples using a microsyringe. Record the amount and lot number of surrogate and matrix spike solutions in the logbook. Pour the water sample into the reservoir, under vacuum; filter as quickly as the vacuum will allow. Drain as much water from the graduated cylinder as possible. Rinse the graduated cylinder once with deionized water and add to the reservoir. Transfer the sample identification tape from the graduated cylinder to the corresponding extraction disk.

After extraction is complete allow the disk to air dry with the vacuum on for at least five minutes.

Remove the extraction disk from the manifold, insert a 40 ml vial into the collection chamber for eluate collection, and replace the extraction disk.

Add 3 ml of acetone, draw into filter with vacuum on and allow the filter to soak for approximately one minute. Add 5 ml of methylene chloride:ethyl acetate (1:1) to the reservoir. Draw 2-3 ml of the solvent through the disk then release the vacuum. Allow the remaining solvent to soak the disk for approximately two minutes then draw remainder of the solvent through the filter under a vacuum.

Repeat the above step twice more with two, 5 ml aliquots of methylene chloride:ethyl acetate (1:1). Prepare sodium sulfate filter funnels by adding a small amount of glass wool to a small glass funnel and adding approximately 25-30 grams of anhydrous sodium sulfate to the funnel. Place a pre-cleaned 40 ml collection vial under the funnel. Remove residual water from the eluate by passing the eluate through the sodium sulfate, collecting the sample in the collection vial. Rinse the first vial with approximately 5 ml of methylene chloride and pass the rinseate through the sodium sulfate. Repeat the vial rinse step. Transfer the sample identification tape to the collection vial. Rinse the sodium sulfate with about 5 ml of methylene chloride. Allow the sodium sulfate to drain completely, then remove the collection vial and cap with a teflon-lined cap prior to sample concentration. If sample concentration is not to proceed immediately, store the extracts at <0°C.

Concentrate the sample extract using nitrogen blowdown to approximately 3-5 ml, as measured by visual observation. Using a Pasteur pipet, transfer the sample to a 15 ml graduated, conical centrifuge tube along with the sample identification tape. Rinse the 40 ml vial with a small amount of methylene chloride and transfer to the centrifuge tube. Repeat the rinse of vial and transfer. Concentrate the sample to 1.0 ml using nitrogen blowdown. Never allow the sample extract to become completely dry.

Label autosampler vials with C18, the sample ID and the date extracted. Spike 10 µl of internal standard solution into the sample and quantitatively transfer the sample to an autosampler vial. Add a septum cap and crimp the cap. Store the extracts at <0°°C until analysis.

Extraction Procedure 2: For acephate and methamidophos

Remove the samples from refrigerator and allow to reach ambient temperature.

Measure 250 ml of sample into a graduated cylinder. Label each cylinder with the appropriate sample ID. Check pH with pH paper. Record the volume and pH in the extraction logbook.

Prepare a method blank and matrix blank with each group of samples extracted. A method blank consists of a 250 ml volume of laboratory deionized water. A matrix blank consists of a 250 ml volume of laboratory potable water. Add approximately 0.1 g of sodium thiosulfate using a small stainless steel scoop and stir until dissolved.

For each sample selected for matrix spike and matrix spike duplicate analysis, measure out two additional 250 ml aliquots. Prepare matrix spikes by adding the 0.25 ml (250 µl) of matrix spike solution to a 250 ml boiling flask using a microsyringe. Record the amount and lot number of the matrix spike solution in the extraction logbook. Evaporate the residue by placing the flasks under a vacuum hood and air drying with the hood on and the sash down.

Assemble the filter apparatus EM-02 using AC-2 cartridges and the 75 ml reservoir.

Condition the cartridge by sequentially eluting approximately 5 ml of acetone, 10 ml of deionized water, 20 ml of 1 N HCl, and 10 ml of deionized water. DO NOT ALLOW THE CARTRIDGE TO GO DRY AT ANY POINT.

Connect the tubing from the peristaltic pumps to the bottom of the 15 ml reservoirs and fill the reservoirs with approximately 10 ml of DI water. After the conditioning step transfer the filters carefully to the extraction apparatus. Cover the top of the 15 ml reservoir with an index finger and with the other hand pull off the tubing and push the cartridge firmly onto the bottom of the reservoir. Replace tubing onto the bottom of the cartridge.

Snap on the tops to the 15 ml reservoirs tightly with the Teflon tubing securely attached.

Add the samples designated for matrix spikes to the 250 ml flasks to dissolve the residue. Swirl the flasks several times to dissolve the residue. Transfer the sample identification tape to the flasks.

Add 250 µl of tributylphosphate surrogate solution to all samples except those designated for matrix spike and matrix spike duplicates.

Place a teflon line from the extraction apparatus into each graduated cylinder and round bottom flask, ensuring that the line goes to the bottom of the cylinders and flasks. Turn on the peristaltic pump. The peristaltic pump has been previously calibrated to provide a flow rate through the extraction cartridges of approximately 2-3 ml per minute. Verify the approximate flow rate by setting a timer and measuring the volume removed from each sample after a period of time, between 1-1.5 hours. If the flow rate is higher than 3.5 ml, the peristaltic pump should be recalibrated prior to further extractions.

After the sample has eluted, rinse the container with 10 ml of deionized water and add to the reservoir. Transfer the sample identification tape from the graduated cylinders and round bottom flasks to the cartridges.

Allow the cartridge to run dry for 2 min.

Remove the cartridge, invert it and connect it to a 10 ml Hamilton (or equivalent) glass syringe with a luer adapter using a short piece of Teflon tubing. Transfer the tape with the sample ID onto the neck of the 50 ml pear shaped flask to which that cartridge will be associated with.

Add 10 ml of acetone to the syringe and elute 3 ml in the opposite direction of the sample flow into a 50 ml pear shaped flask.

Stop the elution and allow the cartridge packing material to soak with acetone for 15 minutes before eluting the remaining volume of acetone. Elute the remaining acetone.

Repeat the elution step with an additional 10 ml aliquot of acetone.

Add 8 ml of ethyl acetate to the eluate and evaporate to dryness on a the Bucchi rotary evaporator. If residual water is present, add an additional 5 ml of ethyl acetate and 10 ml of acetone and re-evaporate.

Add 0.5 ml (500 µl) of acetone to the flasks to dissolve the residue. Swirl the flask to dissolve the residue.

Label autosampler vials with AC2, the sample ID and the date extracted. Spike 5 µl of internal standard solution into the sample and transfer to an autosampler vial for analysis.

Sample Analysis
These are recommended parameters for the Rtx-200 column. These parameters may be adjusted to optimize responses as necessary.

Due to the addition of co-extractives in the chromatographic system during a series of runs, active sites are formed in the GC/MS system, which result in a reduced response to certain pesticide compounds. Consequently, it is necessary to prime the GC/MS system after the analysis of each standard, sample, blank, and matrix spike. This accomplished by injection a system priming mix after each primary injection during a run sequence. The system priming mix used for acephate and methamidophos analysis should contain these compounds at 50 ug/ml in acetone. The system priming mix for diazinon, chlorpyrifos, malathion, guthion and their oxons should contain guthion at 50 ug/ml and guthion oxon at 100 ug/ml.

GC and Detector Conditions for analysis of acephate, methamidophos and tributylphosphate.

Method AC2SIM.M – Appendix A
Initial oven temperature -120 °C
Initial time - 3 minutes
Injection volume - 2 µl
Injector temperature 270 °C
Rate - 20°C/min
Final temperature - 290°C
Final time – 2.00 minutes
Total runtime - 13.5 minutes

GC and Detector Conditions for analysis of azinphos methyl, azinphos methyl oxon, chlorpyrifos, chlorpyrifos oxon, diazinon, diazinon oxon, malathion, malathion oxon, triphenylphosphate

Method C18SIM.M – Appendix B
Initial oven temperature -120 °C
Initial time - 3 minutes
Injection volume - 2 µl
Injector temperature 270 °C
Rate - 25°C/min
Final temperature - 290°C
Final time – 2.20 minutes
Total runtime – 12.0 minutes

See Appendix A and B for complete printed methods containing GC/MS-SIM data acquisition conditions. These conditions may be adjusted as necessary. The method files also contain the data quantitation parameters. The method quantitates and prints a quantitation report.

Acquire data for each sample using the appropriate method file, AC2SIM.M or C18SIM2.M.

The retention time of the GC peak of the quantitation ion for the selected compound of interest needs to be within ± 6 seconds of the average retention time for each compound as determined from the initial calibration.

Mass spectral verification for each selected compound is done by comparing the relative integrated abundance values of the two significant ions monitored with relative integrated abundance values obtained from calibration solutions analyzed initially. The relative ratios of the primary and secondary ions need to be within ± 20 % of the ratios obtained on injection of a standard free of interferences.

7.0 Calculation of Results

The software will calculate the solution concentration in ng/µl injected. The concentration of the sample can be calculated manually by

$$\frac{C_i * A_c * 1000}{RF_c * A_i * V} = C$$

C = Concentration in the sample in µg/L
C_i = Concentration of the internal standard in µg/ml
A_c = Area of the quant ion of the selected compound
A_i = Area of the quant ion of the internal standard
V = volume of the sample in ml
Rf_c = relative response factor for the selected compound

Sample results are reported to 3 significant figures. For rounding significant figures, refer to EASI SOP *GE-06.01: Reporting Data as a Final Result.*

The internal standard acenphthene-d_{10} is used to calculate acephate, methamidophos and tributylphosphate. Phenanthrene-d_{10} is used to calculate diazinon, diazinon oxon, malathion, malathion oxon, chlorpyrifos and chlorpyrifos oxon. Chrysene-d_{12} is used to calculate triphenylphosphate, guthion and its oxon.

8.0 QC Requirements

The data files should be quantitated and the instrument run log should be filled in as soon as possible after the analysis is complete. During a batch sequence, the data files are to be queued for quantitation immediately after analysis, and the run log filled in as the sequence is completed.

Gas chromatographic retention times may not shift more than thirty seconds. If this should occur, corrective action may be necessary. Check for system malfunction.

Check for saturation of peaks above the calibration range. Dilute the extract accordingly and reanalyze.

Calculate the percent surrogate recovery for the surrogate compound. Surrogates are used by the laboratory to facilitate extraction efficiency evaluation only and no criteria have been established.

The maximum holding time before initial sample extraction is 7 days at 4±2 °C. The maximum holding time for final extracts should be 40 days at 0-4 °C.

The analyte specific MDL values is 0.05 for the selected organophosphorus pesticides and their degradates in water.

Method blanks are prepared from deionized water. Matrix blanks are prepared from laboratory potable water. One method blank and one matrix blank is required for every group of 20 samples or each time a group of samples are extracted by the same method whichever is more frequent.

A method blank may not contain more than ½ the PQL for any target compound. When a blank exceeds these limits it is considered to be out of control and the blank and all associated samples must be reextracted or the data must be qualified with a report narrative. The analyst must locate the source of contamination and corrective actions must be taken before data analysis can be continued.

A matrix spike and duplicate are analyzed in order to evaluate the matrix effect of the sample analysis. Matrix spikes and duplicates must be prepared and analyzed each time a group of samples are extracted. Fortified matrix recoveries and relative percent differences are calculated. Matrix recoveries should be between 70 and 120%. The limit for the relative percent difference between spike and duplicate is 40%.

Mass spectrometer tuning criteria. The minimum area for mass 69 ion is1,000,000 area counts. The mass of 69 ion should be 100 percent abundance, mass 219 ion is 40±20 percent, and mass 414 ion is 6.2 ±5.7 percent relative abundance. The mass assignments must be ± 0.15 atomic mass unit for each ion. The mass peak widths must be between 0.45 to 0.59 atomic mass unit measured at ½ the peak height.

Compound	Retention Time (minutes)	Quantitation Ion (m/z)	Confirmation Ion 1 (m/z)	Confirmation Ion 2 (m/z)
Acephate	8.02	136	94	137
Azinphos methyl	10.43	160	132	none
Azinphos methyl oxon	10.55	160	132	none
Chlorpyrifos	7.73	197	199	314
Chlorpyrifos oxon	8.37	197	199	298
Diazinon	6.73	137	179	153
Diazinon oxon	7.24	137	273	288
Malathion	8.16	125	127	173
Malathion oxon	8.28	127	195	173
Methamidophos	6.14	94	141	136

Compound	Retention Time (minutes)	Quantitation Ion (m/z)	Confirmation Ion 1 (m/z)	Confirmation Ion 2 (m/z)
Tributylphosphate	8.35	99	none	none
Triphenylphosphate	9.56	326	none	none
Acenaphthene-d_{10}	6.44	164	none	none
Phenanthrene-d_{10}	6.90	188	none	none
Chrysene-d_{12}	9.71	240	none	none

The retention time of the GC peak of the quantitation ion for the selected compound of interest needs to be within ± 6 seconds of the average retention time for each compound as determined from the initial calibration. When identifying target analytes in a study sample, the peak shape and width will be evaluated manually by visual inspection of the extracted ion profile to determine that they are similar to those in the fortified samples.

Initial calibration data are acceptable if the correlation coefficient, r, is ≥0.99 for linear and the coefficient of the determination, COD, is ≥ 0.99 for non-linear curves calculated across the working concentration range for each compound or surrogate.

Non-compliance: Analytical performance criteria stated in this SOP may not always be achievable in study samples even when corrective actions were employed in an attempt to meet SOP requirements. In certain pressing situations such as holding time near expiring or quick turnaround requirements, it may be necessary to sacrifice some criteria and proceed with the analysis. Such a decision is left to the study director and will be reported to the study director or his designate as soon as possible. All deviations from the SOP must be thoroughly documented and reported to the study director. The study director is the only individual who can approve changes to the study and will direct the issuance of a protocol deviation.

9.0 Safety

Standard laboratory safety precautions should be adhered to at all times. This assumes that all samples are hazardous.

The use of hoods, safety glasses, lab coats, and any other appropriate safety gear is necessary.

MSDSs are available for all chemicals used in this procedure and should be referred to by all analysts.

APPENDICES

Appendix A

Method AC2SIM.M from Chemstation

TOPLEVEL PARAMETERS

Method Information For: C:\HPCHEM\1\METHODS\AC2SIM.M

Method Sections To Run:

( ) Save Copy of Method With Data
( ) Pre-Run Cmd/Macro =
(X) Data Acquisition
(X) Data Analysis
( ) Post-Run Cmd/Macro =

Method Comments:
This is the SIM method for Acephate and Methamidophos.

END OF TOPLEVEL PARAMETERS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet: GC
Injection Source: GC ALS
Mass Spectrometer: Enabled

HP GC Injector

Front Injector:
No parameters specified

Back Injector:

Sample Washes	1	
Sample Pumps	4	
Injection Volume	2.0	microliters
Syringe Size	10.0	microliters
On Column	Off	
Nanoliter Adapter	Off	
PostInj Solvent A Washes	3	
PostInj Solvent B Washes	3	
Viscosity Delay	0	seconds
Plunger Speed	Fast	

HP5890 Temperature Parameters

Zone Temperatures:	State	Setpoint
Inlet A:	Off	50 C
Inlet B:	On	270 C
Detector A:	Off	50 C
Detector B:	On	290 C

Method: AC2SIM.M Tue Aug 03 17:21:24 1999 Page: 1

Auxiliary: Off 50 C

Oven Parameters:
Oven Equib Time: 0.50 minutes
Oven Max: 300 C
Oven State: On
Cryo State: Off
Cryo Blast: Off
Ambient: 25 C

Oven Program:
Initial Temperature: 120 C
Initial Time: 3.00 minutes

Level	Rate (C/minute)	Final Temperature (C)	Final Time (minutes)
1	20.0	290	2.00
2(A)	0.0	0	0.00
3(B)	0.0	0	0.00

Next Run Time: 13.50 minutes

HP5890 Inlet Pressure Programs

GC Pressure Units: kPa

Inlet A:
Constant Flow: Off
Constant Flow Pressure: 0 kPa
Constant Flow Temperature: 50 C
Initial Pressure: 0 kPa
Initial Time: 650.00 minutes

Level	Rate (kPa/minute)	Final Pressure (kPa)	Final Time (minutes)
1	0.0	0	0.00
2(A)	0.0	0	0.00
3(B)	0.0	0	0.00

Total Program Time: 650.00 minutes

Column Length: 30.00 m
Column Diameter: 0.530 mm
Gas: He
Vacuum Compensation: Off

Inlet B:
Constant Flow: Off
Constant Flow Pressure: 207 kPa
Constant Flow Temperature: 120 C
Initial Pressure: 138 kPa
Initial Time: 650.00 minutes

Level	Rate (kPa/minute)	Final Pressure (kPa)	Final Time (minutes)
1	0.0	0	0.00
2(A)	0.0	0	0.00
3(B)	0.0	0	0.00

Total Program Time: 650.00 minutes

Column Length:	15.00 m
Column Diameter:	0.250 mm
Gas:	He
Vacuum Compensation:	On

HP5890 Packed Column Flow Control

Inlet A not used to control packed column flow.

Inlet B not used to control packed column flow.

HP5890 Purge Valve Settings

Inlet Purge	Init Value	On Time	Off Time	Splitless Injection
A	Off	1.00	0.00	No
B	Off	0.75	0.00	Yes

HP5890 Valve and Relay Information

Initial Setpoints:

5890 Valves:

Valve 1: Off	Valve 2: Off	Valve 3: Off	Valve 4: Off

19405 Valves:

Valve 5: Off	Valve 6: Off	Valve 7: Off	Valve 8: Off

19405 Relays:

Relay 1: Off	Relay 2: Off	Relay 3: Off	Relay 4: Off

HP5890 Detector Information

Detector	Type	State
A	---	Off
B	---	Off

HP5890 Signal Information

Not saving signal data.

Signal	Source	Peak Width	Data Rate	Start Data	Stop Data
1	Testplot	0.053	5.000	0.00	1.00
2	Testplot	0.053	5.000	0.00	1.00

MS ACQUISITION PARAMETERS

General Information
------- -----------

Tune File : high.u
Acquistion Mode : SIM

Method: AC2SIM.M Tue Aug 03 17:21:24 1999 Page: 3

MS Information
-- -----------

Solvent Delay : 5.00 min

EM Absolute : False
EM Offset : 0
Resulting EM Voltage : 2435.3

[Sim Parameters]

GROUP 1
Group ID : 1
Resolution : Low
Group Start Time : 0.00
Plot 1 Ion : 164.0

Ions/Dwell In Group	(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)
	(164.0,	70)	(141.0,	70)	(136.0,	70)
	(94.0,	70)	(99.0,	70)		

END OF MS ACQUISITION PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

DATA ANALYSIS PARAMETERS

Method Name: C:\HPCHEM\1\METHODS\AC2SIM.M

Percent Report Settings

Sort By: Signal

Output Destination
Screen: No
Printer: Yes
File: No

Integration Events: Meth Default

Generate Report During Run Method: No

Signal Correlation Window: 0.020

Qualitative Report Settings

Peak Location of Unknown: Apex

Library to Search	Minimum Quality
DEMO.L	0

Integration Events: Meth Default

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: No

Quantitative Report Settings

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: Yes

Acephate and Methamidophos Analysis
Calibration Last Updated: Fri Jun 04 16:55:54 1999

Reference Window: 2.00 Minutes
Non-Reference Window: 1.00 Minutes
Correlation Window: 0.10 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information

---

1) Acenaphthene-d10 (ISTD)

Ret. Time 6.40 min., Extract & Integrate from 5.90 to 6.90 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 164.00			*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		0.500	209843
2		0.500	256832

3	0.500	195575
4	0.500	185679
5	0.500	188337
6	0.500	188077

Qualifier Peak Analysis ON ISTD conc: 0.500 ppm
Curve Fit: Linear

---

2) Methamidophos ( )

Ret. Time 6.09 min., Extract & Integrate from 5.59 to 6.59 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 94.00			*** METH DEFAULT ***
Q1 141.00	19.30	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	178406
2		0.500	114201
3		0.250	31367
4		0.100	6951
5		0.025	1362
6		0.050	663

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

3) Acephate ( )

Ret. Time 8.00 min., Extract & Integrate from 7.50 to 8.50 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 136.00			*** METH DEFAULT ***
Q1 94.00	84.50	20.0	*** METH DEFAULT ***
Q2 141.00	0.80	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	61089
2		0.500	41087
3		0.250	10674
4		0.100	3055
5		0.025	1678
6		0.050	308

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

4) Tributylphosphate ( )

Ret. Time 8.34 min., Extract & Integrate from 7.84 to 8.84 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 99.00			*** METH DEFAULT ***

Lvl	ID	Conc (ppm)	Response
1		1.000	831758
2		0.500	536103

3	0.250	161853
4	0.100	55144
5	0.025	27509
6	0.050	13754

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

END OF DATA ANALYSIS PARAMETERS

Appendix B

Method PSIM.M from Chemstation

TOPLEVEL PARAMETERS

Method Information For: C:\HPCHEM\1\METHODS\C18SIM.M

Method Sections To Run:

( ) Save Copy of Method With Data
( ) Pre-Run Cmd/Macro =
(X) Data Acquisition
(X) Data Analysis
( ) Post-Run Cmd/Macro =

Method Comments:
This is the SIM method for Azinphos methyl, Chlorpyrifos, Daizinon, Malathion and their oxons

END OF TOPLEVEL PARAMETERS

INSTRUMENT CONTROL PARAMETERS

Sample Inlet: GC
Injection Source: GC ALS
Mass Spectrometer: Enabled

HP GC Injector

Front Injector:
No parameters specified

Back Injector:

Sample Washes	1
Sample Pumps	4
Injection Volume	2.0 microliters
Syringe Size	10.0 microliters
On Column	Off
Nanoliter Adapter	Off
PostInj Solvent A Washes	3
PostInj Solvent B Washes	3
Viscosity Delay	0 seconds
Plunger Speed	Fast

HP5890 Temperature Parameters

Zone Temperatures:	State	Setpoint
Inlet A:	Off	50 C
Inlet B:	On	270 C
Detector A:	Off	50 C

Detector B: On 290 C
Auxiliary: Off 50 C

Oven Parameters:
Oven Equib Time: 0.50 minutes
Oven Max: 300 C
Oven State: On
Cryo State: Off
Cryo Blast: Off
Ambient: 25 C

Oven Program:
Initial Temperature: 120 C
Initial Time: 3.00 minutes

Level	Rate (C/minute)	Final Temperature (C)	Final Time (minutes)
1	25.0	290	2.20
2(A)	0.0	50	1.00
3(B)	0.0	50	1.00

Next Run Time: 12.00 minutes

HP5890 Inlet Pressure Programs

GC Pressure Units: psi

Inlet A:
Constant Flow: Off
Constant Flow Pressure: 0.0 psi
Constant Flow Temperature: 50 C
Initial Pressure: 0.0 psi
Initial Time: 650.00 minutes

Level	Rate (psi/minute)	Final Pressure (psi)	Final Time (minutes)
1	0.00	0.0	0.00
2(A)	0.00	0.0	0.00
3(B)	0.00	0.0	0.00

Total Program Time: 650.00 minutes

Column Length: 30.00 m
Column Diameter: 0.530 mm
Gas: He
Vacuum Compensation: Off

Inlet B:
Constant Flow: Off
Constant Flow Pressure: 30.0 psi
Constant Flow Temperature: 120 C
Initial Pressure: 20.0 psi
Initial Time: 650.00 minutes

Level	Rate (psi/minute)	Final Pressure (psi)	Final Time (minutes)
1	0.00	0.0	0.00
2(A)	0.00	0.0	0.00
3(B)	0.00	0.0	0.00

Total Program Time: 650.00 minutes

Method: C18SIM.M Tue Aug 03 17:20:14 1999 Page: 2

Column Length:	15.00 m
Column Diameter:	0.250 mm
Gas:	He
Vacuum Compensation:	On

HP5890 Packed Column Flow Control

Inlet A not used to control packed column flow.

Inlet B not used to control packed column flow.

HP5890 Purge Valve Settings

Inlet Purge	Init Value	On Time	Off Time	Splitless Injection
A	On	0.00	0.00	No
B	Off	0.75	0.00	Yes

HP5890 Valve and Relay Information

Initial Setpoints:

5890 Valves:

Valve 1: Off	Valve 2: Off	Valve 3: On	Valve 4: Off

19405 Valves:

Valve 5: Off	Valve 6: Off	Valve 7: Off	Valve 8: Off

19405 Relays:

Relay 1: Off	Relay 2: Off	Relay 3: Off	Relay 4: Off

HP5890 Detector Information

Detector	Type	State
A	---	Off
B	---	Off

HP5890 Signal Information

Not saving signal data.

Signal	Source	Peak Width	Data Rate	Start Data	Stop Data
1	Testplot	0.053	5.000	0.00	1.00
2	Testplot	0.053	5.000	0.00	1.00

MS ACQUISITION PARAMETERS

General Information
------- -----------

Tune File : high.u

Method: C18SIM.M Tue Aug 03 17:20:14 1999 Page: 3

Acquistion Mode : SIM

MS Information
-- -----------

Solvent Delay : 6.00 min

EM Absolute : False
EM Offset : 200
Resulting EM Voltage : 2635.3

[Sim Parameters]

GROUP 1
Group ID : 1
Resolution : Low
Group Start Time : 0.00
Plot 1 Ion : 137.0

Ions/Dwell In Group	(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)
	(137.0,	70)	(188.0,	70)	(153.0,	70)
	(179.0,	70)	(273.0,	70)	(288.0,	70)

GROUP 2
Group ID : 2
Resolution : Low
Group Start Time : 7.60
Plot 1 Ion : 127.0

Ions/Dwell In Group	(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)
	(127.0,	70)	(125.0,	70)	(173.0,	70)
	(195.0,	70)	(197.0,	70)	(199.0,	70)
	(298.0,	70)	(314.0,	70)		

GROUP 3
Group ID : 3
Resolution : Low
Group Start Time : 9.00
Plot 1 Ion : 132.0

Ions/Dwell In Group	(Mass,	Dwell)	(Mass,	Dwell)	(Mass,	Dwell)
	(132.0,	70)	(160.0,	70)	(326.0,	70)
	(240.0,	70)				

END OF MS ACQUISITION PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

DATA ANALYSIS PARAMETERS

Method Name: C:\HPCHEM\1\METHODS\C18SIM.M

Percent Report Settings

Sort By: Signal

Output Destination
    Screen: No
    Printer: Yes
    File: No

Integration Events: Meth Default

Generate Report During Run Method: No

Signal Correlation Window: 0.020

Qualitative Report Settings

Peak Location of Unknown: Apex

Library to Search	Minimum Quality
DEMO.L	0

Integration Events: Meth Default

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: No

Quantitative Report Settings

Report Type: Summary

Output Destination
    Screen: No
    Printer: Yes
    File: No

Generate Report During Run Method: Yes

Organophshorus Pesticide Analysis
Calibration Last Updated: Mon Jul 19 00:31:38 1999

Reference Window: 1.00 Minutes
Non-Reference Window: 0.50 Minutes
Correlation Window: 0.10 minutes
Default Multiplier: 1.00
Default Sample Concentration: 0.00

Compound Information

---

1) Phenanthrene-d10 (ISTD)

Ret. Time 6.88 min., Extract & Integrate from 6.38 to 7.38 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 188.00			*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		0.500	940404
3		0.500	1033453
4		0.500	974080
5		0.500	865274
2		0.500	910460

Qualifier Peak Analysis ON ISTD conc: 500.000 pg
Curve Fit: Linear, forced through origin

---

2) Diazinon ( )

Ret. Time 6.71 min., Extract & Integrate from 6.21 to 7.21 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 179.00			*** METH DEFAULT ***
Q1 137.00	143.70	20.0	*** METH DEFAULT ***
Q2 153.00	48.80	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	228176
3		0.250	61804
4		0.100	23655
5		0.050	11508
2		0.500	110236

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

3) Diazinon O analog ( )

Ret. Time 7.23 min., Extract & Integrate from 6.73 to 7.73 min.

Signal	Rel Resp.	Pct. Unc.(rel)	Integration
Tgt 273.00			*** METH DEFAULT ***
Q1 288.00	0.00	20.0	*** METH DEFAULT ***
Q2 137.00	145.80	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	283751
3		0.250	74036

4	0.100	28093
5	0.050	13141
2	0.500	134859

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

4) Chlorpyrifos ( )

Ret. Time 7.71 min., Extract & Integrate from 7.21 to 8.21 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	197.00			*** METH DEFAULT ***
Q1	199.00	92.90	20.0	*** METH DEFAULT ***
Q2	314.00	0.00	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	304204
3		0.250	70171
4		0.100	28400
5		0.050	33092
2		0.500	124532

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

5) Malathion ( )

Ret. Time 8.14 min., Extract & Integrate from 7.64 to 8.64 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	173.00			*** METH DEFAULT ***
Q1	125.00	136.80	20.0	*** METH DEFAULT ***
Q2	127.00	103.10	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	328384
3		0.250	84671
4		0.100	31155
5		0.050	14973
2		0.500	153805

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

6) Malathion O analog ( )

Ret. Time 8.27 min., Extract & Integrate from 7.77 to 8.77 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	195.00			*** METH DEFAULT ***
Q1	173.00	72.00	20.0	*** METH DEFAULT ***
Q2	127.00	797.40	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	71418
3		0.250	18906

4	0.100	7916
5	0.050	621
2	0.500	33939

Qualifier Peak Analysis ON
Curve Fit: Quadratic

---

7) Chlorpyrifos O analog ( )

Ret. Time 8.36 min., Extract & Integrate from 7.86 to 8.86 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	199.00			*** METH DEFAULT ***
Q1	197.00	118.50	20.0	*** METH DEFAULT ***
Q2	298.00	56.10	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	124688
3		0.250	32993
4		0.100	13421
5		0.050	5885
2		0.500	58433

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

8) Chrysene-d12 (ISTD)

Ret. Time 9.69 min., Extract & Integrate from 9.19 to 10.19 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	240.00			*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		0.500	850097
3		0.500	915699
4		0.500	777010
5		0.500	704033
2		0.500	767633

Qualifier Peak Analysis ON ISTD conc: 500.000 pg
Curve Fit: Linear, forced through origin

---

9) Triphenylphosphate ( )

Ret. Time 9.54 min., Extract & Integrate from 9.04 to 10.04 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	326.00			*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	432935
3		0.250	115691
4		0.100	40469
5		0.050	22026
2		0.500	201777

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

10) Guthion ( )

Ret. Time 10.41 min., Extract & Integrate from 10.31 to 10.51 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	160.00			*** METH DEFAULT ***
Q1	132.00	93.20	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	275074
3		0.250	62574
4		0.100	17273
5		0.050	9768
2		0.500	101278

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

---

11) Guthion O analog ( )

Ret. Time 10.53 min., Extract & Integrate from 10.43 to 10.63 min.

Signal		Rel Resp.	Pct. Unc.(rel)	Integration
Tgt	160.00			*** METH DEFAULT ***
Q1	132.00	106.70	20.0	*** METH DEFAULT ***

Lvl	ID	Conc (pg)	Response
1		1.000	424458
3		0.250	88637
4		0.100	24468
5		0.050	10597
2		0.500	136503

Qualifier Peak Analysis ON
Curve Fit: Quadratic, forced through origin

END OF DATA ANALYSIS PARAMETERS

Appendix C

Chromatograms

Quantitation Report (Not Reviewed)

Data File : C:\HPCHEM\1\DATA\072099\OP1804.D Vial: 1
Acq On : 20 Jul 1999 11:53 pm Operator:
Sample : 1000pg am std Inst : GC/MS Ins
Misc : Multiplr: 1.00
MS Integration Params: rteint.p
Quant Time: Jul 21 0:05 1999 Quant Results File: P0720.RES

AC25IM.mg 6-3-99

Quant Method : C:\HPCHEM\1\METHODS\~~P0720.M~~ (RTE Integrator)
Title : Acephate and Methamidophos Analysis
Last Update : Tue Jul 20 22:57:02 1999
Response via : Initial Calibration
DataAcq Meth : P0720

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)
1) Acenaphthene-d10	4.67	164	291696	0.50	ppm	-0.01
System Monitoring Compounds						
Target Compounds						Qvalue
2) Methamidophos	4.26	94	295457	1.15	ppm	# 73
3) Acephate	6.20	136	132526	1.49	ppm	91
4) Tributylphosphate	6.56	99	1417508	1.20	ppm	100

(#) = qualifier out of range (m) = manual integration
OP1804.D P0720.M Wed Jul 21 00:05:29 1999 RPT1 Page 1

Quantitation Report

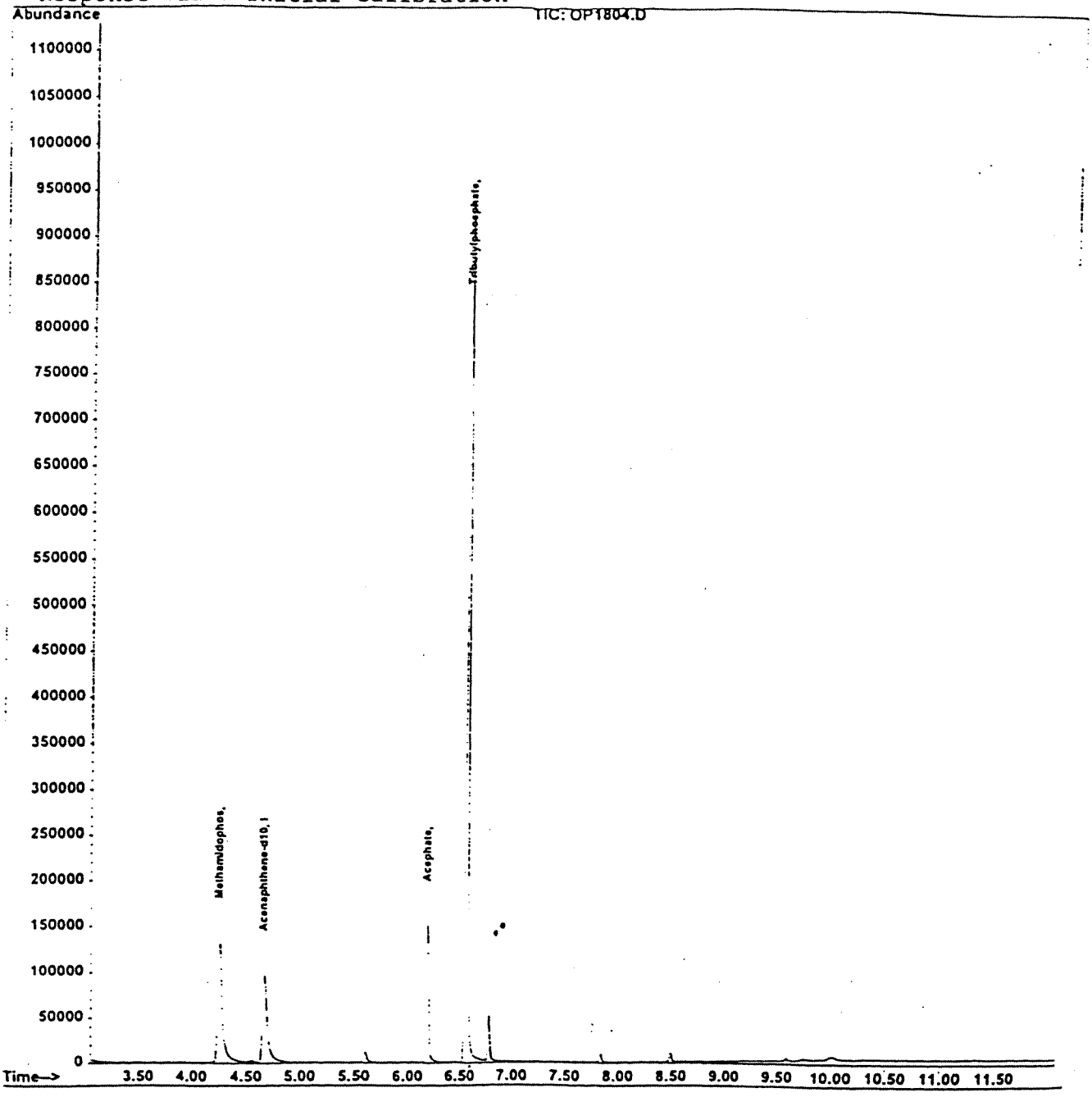
Data File : C:\HPCHEM\1\DATA\072099\OP1804.D
Acq On : 20 Jul 1999 11:53 pm
Sample : 1000pg am std
Misc :
MS Integration Params: rteint.p
Quant Time: Jul 21 0:05 1999

Vial: 1
Operator:
Inst : GC/MS Ins
Multiplr: 1.00

Quant Results File: P0720.RES

AC25IM.M 8-3-99

Method : C:\HPCHEM\1\METHODS\~~P0720.M~~ (RTE Integrator)
Title : Acephate and Methamidophos Analysis
Last Update : Tue Jul 20 22:57:02 1999
Response via : Initial Calibration



OP1804.D P0720.M Wed Jul 21 00:05:29 1999 RPT1 Page 2

Quantitation Report (Not Reviewed)

Data File : C:\HPCHEM\1\DATA\080199\OP3078.D Vial: 1
Acq On : 1 Aug 1999 8:54 pm Operator:
Sample : 1000pg opc std Inst : GC/MS Ins
Misc : Multiplr: 1.00
MS Integration Params: rteint.p
Quant Time: Aug 1 21:06 1999 Quant Results File: P0722.RES

C18 Sim. M 8-3-99
Quant Method : C:\HPCHEM\1\METHODS\~~P0722.M~~ RTE Integrator)
Title : Organophshorus Pesticide Analysis
Last Update : Mon Jul 19 00:31:38 1999
Response via : Initial Calibration
DataAcq Meth : P0722

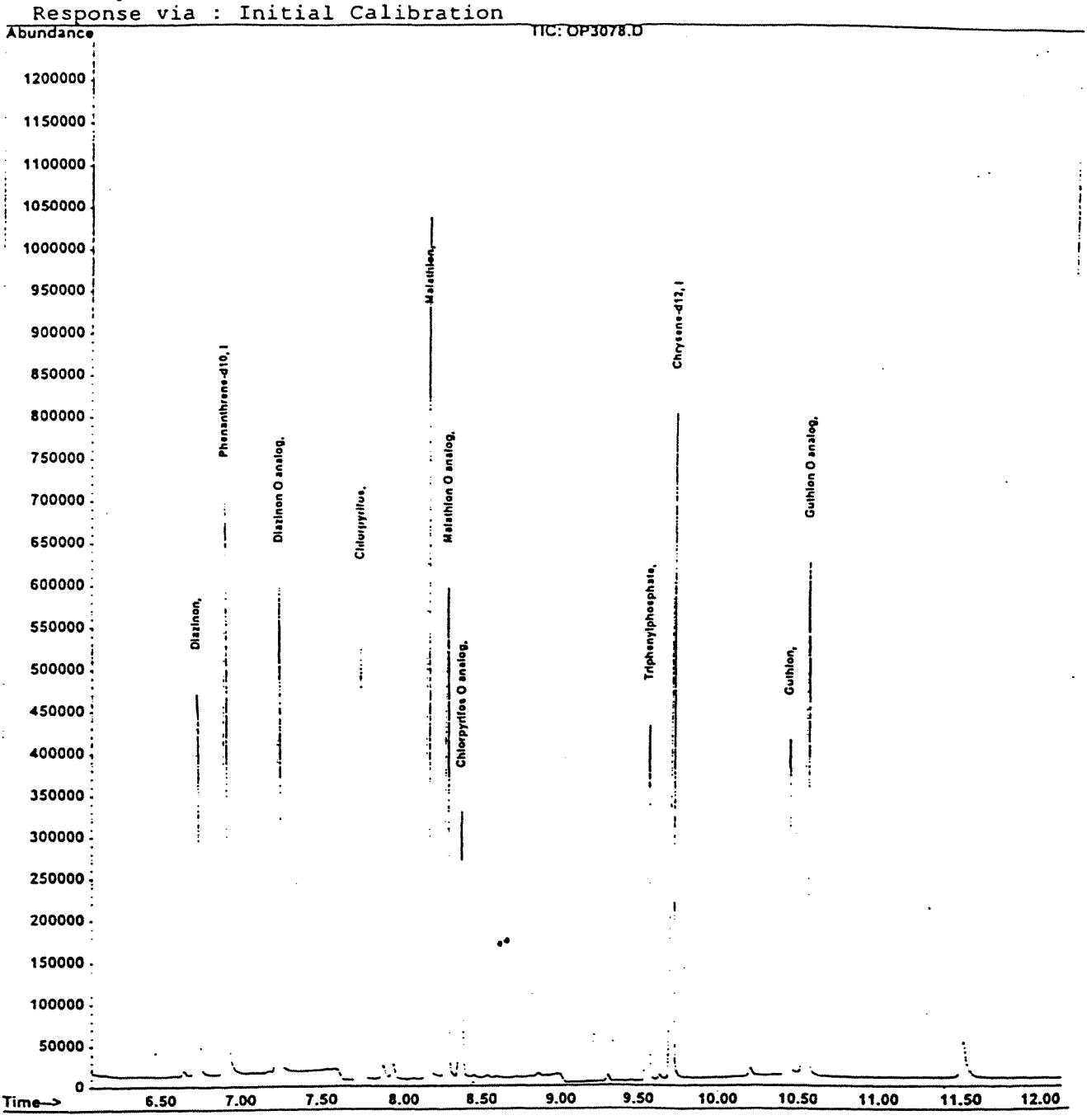
Internal Standards	R.T.	QIon	Response	Conc	Units		Dev(Min)
1) Phenanthrene-d10	6.89	188	1088653	500.00	pg		0.00
8) Chrysene-d12	9.71	240	976290	500.00	pg		0.00
System Monitoring Compounds							
Target Compounds							Qvalue
2) Diazinon	6.73	179	263543	1001.12	pg		83
3) Diazinon O analog	7.24	273	309060	998.94	pg	#	76
4) Chlorpyrifos	7.73	197	281314	1001.89	pg	#	97
5) Malathion	8.16	173	374409	1000.30	pg		92
6) Malathion O analog	8.28	195	78670	997.82	pg		72
7) Chlorpyrifos O analog	8.37	199	139903	998.18	pg	#	90
9) Triphenylphosphate	9.56	326	494670	1000.07	pg		100
10) Guthion	10.44	160	307210	998.39	pg		98
11) Guthion O analog	10.54	160	461631	997.42	pg		89

(#) = qualifier out of range (m) = manual integration
OP3078.D P0722.M Sun Aug 01 21:06:17 1999 RPT1 Page 1

Quantitation Report

Data File : C:\HPCHEM\1\DATA\080199\OP3078.D Vial: 1
Acq On : 1 Aug 1999 8:54 pm Operator:
Sample : 1000pg opc std Inst : GC/MS Ins
Misc : Multiplr: 1.00
MS Integration Params: rteint.p
Quant Time: Aug 1 21:06 1999 Quant Results File: P0722.RES

C18Sim.M 5-349
Method : C:\HPCHEM\1\METHODS\~~P0722.M~~ (RTE Integrator)
Title : Organophshorus Pesticide Analysis
Last Update : Mon Jul 19 00:31:38 1999
Response via : Initial Calibration



OP3078.D P0722.M Sun Aug 01 21:06:18 1999 RPT1 Page 2

ORGANOPHOSPHORUS PESTICIDES

Compound	Retention Time (minutes)	Quantitation Ion (m/z)	Confirmation Ion 1 (m/z)	Confirmation Ion 2 (m/z)
Acephate	8.02	136	94	137
Azinphos methyl	10.43	160	132	none
Azinphos methyl oxon	10.55	160	132	none
Chlorpyrifos	7.73	197	199	314
Chlorpyrifos oxon	8.37	197	199	298
Diazinon	6.73	137	179	153
Diazinon oxon	7.24	137	273	288
Malathion	8.16	125	127	173
Malathion oxon	8.28	127	195	173
Methamidophos	6.14	94	141	136
Tributylphosphate	8.35	99	none	none
Triphenylphosphate	9.56	326	none	none
Acenaphthene-d_{10}	6.44	164	none	none
Phenanthrene-d_{10}	6.90	188	none	none
Chrysene-d_{12}	9.71	240	none	none

PROTOCOL AMENDMENT FORM

AMENDMENT NUMBER: 2

Protocol: En-fate Study No. 00102

Protocol Title: CHLORINE DEGRADATION OF SELECTED ORGANOPHOSPHORUS PESTICIDES AND CERTAIN OF THEIR DEGRADATES IN A DRINKING WATER MATRIX

Compound/Formulation: Acephate and Methamidophos.

AMENDMENT(S):

1) SECTION 6: ANALYTICAL METHODOLOGY

CHANGES: Analytical methodologies for the above referenced compounds will be conducted as per SOP NO.: EASI/GLP_ MS-20.04. (Changing analytical methodology from GC/MS –SIM to GC/FPD.

REASON(S)Analysis of acephate and methamidophos by GC/MS-SIM results in false positive results due to the presence of quantitation ion 141 from matrix interferences. Additionally, analyte recovery from fortified samples results in abnormally high recoveries and poor precision. Studies conducted on fortified samples using GC/FPD have documented improved analyte recovery and precision. Sensitivity is also much improved.

EFFECT OF CHANGE: Sample extraction procedures will not be affected and analyte recovery and precision will improve. Modification of the Standard Operating Procedure, EASI.GLP_MS-20.04, will be required to include analysis of sample extracts by GC/FPD.

2) START AND TERMINATION DATE

CHANGES: The start date for the acephate and methamidophos chlorination study will commence April 3, 2000. Anticipated termination date is May 30, 2000.

REASON(S)Change of analytical methodology for acephate and methamidophos resulted in the experiment being re-run.

EFFECT OF CHANGE: Analyte recovery and precision will improve. Modification of the Standard Operating Procedure, EASI.GLP_MS-20.04, will be required to include analysis of sample extracts by GC/FPD.

Effective date of this Amendment: April 3, 2000

STUDY DIRECTOR: _____ DATE 4/3/2000

Amendments to be distributed per Protocol Distribution List

COPY

THIS PAGE LEFT INTENTIONALLY BLANK

THIS PAGE LEFT INTENTIONALLY BLANK

ENVIRONMENTAL ANALYTICAL SOLUTIONS, INC.

STANDARD OPERATING PROCEDURES

SOP Number: EASI / GLP_MS-20.04

Title: Analysis of Acephate and Methamidophos by GC/FPD

Department: Administrative

Original Author: V. Culpepper Date: 03/01/00

Last Revision: ORIGINAL

Technical Review and Approval: _____ Date: 3-1-00

Mike Antoine
Manager of Laboratory Operations

Master Location: Administrative / QA Office

Other Copies Located: A) ____GC; Extractions_____

_____

This is the Standard Operating Procedure (SOP) for the analysis of acephate and methamidophos by GC/FPD. Personnel performing this procedure must read, understand and follow it explicitly.

Any changes to this SOP must be made in accordance with SOP EASI/GE01.01 *- GENERATION and REVISION OF STANDARD OPERATING PROCEDURES.*

EXACT COPY OF ORIGINAL

_____ 3.26.00

Signature **Date**

Page 1 **of** 26 total

References:

USGS Open-File Report 95-181 "Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Determination of Pesticides in Water by C-18 Solid-Phase Extraction and Capillary-Column Gas Chromatography/Mass Spectrometry with Selected-Ion Monitoring", 1995

US EPA Test Methods for Evaluating Solid Waste, SW-846, 3rd edition, Method 8141A.

US EPA 40 CFR Part 136, Appendix B. "Definition and Procedure for the Determination of the Method Detection Limit"

US EPA Method 1618: Organo-halide Pesticides, Organo-phosphorus Pesticides, and Phenoxy-acid Herbicides by Wide Bore Capillary column Gas Chromatography with selective Detectors. July 1989.

Holding Time:

All samples must be extracted within 7 days of collection and sample extracts should be analyzed within 40 days of extraction. Disposal of samples will be only with the approval of the study director.

Preservation:

Sample container must contain sodium thiosulfate at approximately 0.01% to quench the redox potential of any residual chlorine or chloramine that may be added by a community water system. All samples must be protected from light and refrigerated at 4°C ± 2°C from the time of collection until extraction.

Sampling:

For water samples, 250 mL of water is required for extraction and should be collected in sufficient volume for a second analysis, i.e. ≥ 500 mL. Samples must be collected in amber glass containers.

1.0 Scope and application

This method covers the determination of acephate and methamidophos. This SOP covers sample preparation and analysis. The analytical method was designed to analyze water samples for the presence of acephate and methamidophos.

The following compounds (target analytes) are determined by this method:

COMPOUND	CAS No.[a]	MDL(ppb)[b]	PQL(ppb)[b]
Acephate	30560-19-1	[reserved]	[reserved]
Methamidophos	10265-92-6	[reserved]	[reserved]

[a]: Chemical Abstracts Service Number
[b]: Method Detection Limit and Practical Quantitation Limit as determined by the laboratory upon spiking drinking water from a local treatment facility.

Detection limits of this method are dependent upon the levels of interferences and instrumental limitations. The limits in the table above typify the minimum quantities that can be detected in water treatment facility effluents. The practical quantitation limit (PQL) is generally accepted as 5 times the MDL. The MDL is determined by multiplying the standard deviation of ≥ 7 analyses by the student t value appropriate for that number of analyses (n-1) at the 99% confidence level.

2.0 Summary of Method

Solid phase extraction procedures are employed for aqueous samples. Analysis is accomplished by injection of a fixed volume of an extract onto a gas chromatographic column equipped with a fused silica capillary column and detection using a flame photometric detector.

3.0 Interferences

Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of analysis by running laboratory reagent blanks.

All reagents are to be tested prior to use to ensure that interferences do not affect analyses.

4.0 Apparatus and Materials

Gas Chromatograph - Hewlett Packard 5890 Series II.

Autosampler, autoinjector - Hewlett Packard 7673.

Chemstation data system

Flame Photometric Detector

Column
Restek-Rtx-200, 30 m length x 0.53 mm inner diameter x 1.0 µm film thickness Restek # 15055 or equivalent

Rotary evaporator Bucchi Model # R-3000 or equivalent

Autosampler vials with teflon-lined crimp top seals

Vacuum extraction apparatus for eluting multiple sorbent cartridges (laboratory constructed) with Masterflex peristaltic pump or equivalent

Waters Sep-Pak Plus AC-2 cartridges Waters Custom # WAT020585 (ref#JJAN20229) or equivalent

SPE Polyethylene Reservoirs, 75 ml Baker # 7120-03 or equivalent

SPE Polyethylene Reservoirs, 15 ml Baker with snap tops and 1.5' of 1/16" i.d., 1/8" o.d. teflon tubing attached to snap tops

pH paper - range of 1-12

Pasteur pipettes, disposable borosilicate glass - 5.75" and 9"

Microsyringes - 10 µl, 25 µl, 50 µl, 100 µl, 250 µl, 500 µl, 5 ml and 10 ml, Hamilton models or equivalent

Class A Graduated cylinders – 1000 ml and 250 ml capacity

Class A Volumetric flasks – 10.0 ml, 25.0 ml, 50.0 ml, and 100 ml

40 ml precleaned vials

250 ml round bottom flasks

4.0 Apparatus and Materials, cont.

50 ml pear shaped evaporation flasks with 24/40 joint

Analytical balance - capable of accurately weighing 10 g ± 0.0001g, Denver Instruments Model A-250 or equivalent

Small stainless steel spatulas

5.0 Reagents and Standards

Chemicals and Reagents

Acephate - Valent U.S.A. Corporation Lot # AS 40p or equivalent

Methamidophos - Bayer Corporation Lot # K-753 or equivalent

Organic free water - carbon filtered, deionized water

Laboratory potable water (for matrix spikes where applicable)

Hydrochloric acid 1N – Prepared by adding 80 ml of conc. HCl to 880 ml of deionized water

Acetone - ACS reagent grade

Methanol - ACS reagent grade

Methylene Chloride - ACS reagent grade

Helium carrier gas, ultrapure

Nitrogen gas

Stock standard solutions

The preparation of all standards will be documented in the organics standards logbook. Each entry is to be signed and dated by the analyst. The entry should contain adequate information as to how the standard was prepared and how it should be used. The standards should be labeled using the number of the standard logbook and applicable page number to facilitate traceability as well as a short description of the standard, the concentration and the expiration date. Due to the small vials used for some calibration standards, the concentration may be eliminated from the label if the

standard, concentration is known by the label as a working standard and the concentration is traceable to the logbook using the standard I.D.

Stock standards shall be prepared from analytical standards supplied by and characterized in accordance with FIFRA GLPs by the sponsor. It is the responsibility of the sponsor to maintain adequate documentation that verifies compound purity, concentration and identity. Any test/control/reference substances used in the study must be characterized prior to its use in the study. The laboratory will maintain copies of sponsor GLP-certification information in the neat standards logbook.

The surrogate compounds are not characterized in accordance with FIFRA GLPs.

If the standards are prepared in the lab care must be exercised to prevent contamination. Glassware must be scrupulously clean. Use high quality solvents and reference materials assayed at 97% or greater purity.

Calibrate the balance according to the balance SOP. Prepare stock standards by accurately weighing the neat compound to the nearest 0.0001g. Place the volumetric flask to be used on the balance, tare the balance and quantitatively transfer the compound to the flask using a small stainless steel spatula.. The mass of compound to be weighed is dependent upon the amount of standard available and the size of the volumetric dilution flask. When possible, use a 10.0 ml volumetric flask for preparation of stock standards. When possible, i.e. when the quantity of neat standard is sufficient, prepare standards to provide a final concentration of approximately 10,000 ug/ml. For liquid neat standards, use an appropriate microsyringe for optimal control of standard addition during weighing and quantitatively transfer to the tared volumetric flask. Dilute stock standards to volume with ACS-grade acetone. Stocks may be prepared as single components or as mixtures.

Store stock standards in glass screw top vials with Teflon septa. Store at <0°C. Standards may be stored up to 1 year unless the standards show signs of degradation.

Prepare working standard solutions from stock or intermediate standards for direct analysis on the GC as follows:

Prepare 1.0 ml each of 6 working standards by diluting (adding) the appropriate amounts of the stock standard solutions in matrix-amended acetone (depending on the exact concentration of the stock or intermediate standard) to give the following concentrations: 0.0125, 0.025, 0.050, 0.100, 0.200, and 0.300 µg/ml. The 0.0125 µg/ml standard is included in the run sequence and analyzed for verification of instrument sensitivity. Matrix-amended acetone is prepared by extraction of deionized water exactly as described for sample extraction in Section 6.0 Sample Preparation. The resulting 0.5 ml of matrix-amended acetone is then used for standard preparation.

All working standards are prepared in 1.0 ml crimp top ALS vials and must be stored at <0°C. Working standard solutions must be crimped immediately after use and may be stored up to one month unless standards show signs of degradation.

Surrogate Standards

The surrogate used is tributylphosphate. The surrogate solution is spiked prior to extraction using a 0.20 ug/ml working solution (prepared as described below).

Prepare a stock solution by weighing 0.100g of tributylphosphate and dissolving in methylene chloride and bringing to volume in a 10.0 ml volumetric flask. Prepare a 50 µg/ml standard solution by diluting 0.050 ml (50 µl) of this stock solution to 10.0 ml in a volumetric flask with acetone. Prepare a 0.20 µg/ml surrogate working solution by diluting 0.10 ml (100 µl) of the 50 µg/ml solution to 25.0 ml in a volumetric flask with acetone. Spike 0.25 ml (250 µl) into each 250 ml aliquot of sample to be extracted

Pesticide Matrix Spike Solution

Prepare a matrix spiking solution containing the surrogate compounds as follows.

Prepare a stock solution by weighing 0.100g of each neat compound and the surrogate tributylphosphate and dissolving in methylene chloride and bringing to volume in a 10.0 ml volumetric flask. Prepare a 50 µg/ml intermediate standard solution by diluting 0.050 ml (50 µl) of this stock solution to 10.0 ml in a volumetric flask with acetone. Prepare a 0.20 µg/ml working solution by diluting 0.10 ml (100 µl) of the 50 µg/ml solution to 25.0 ml in a volumetric flask with acetone. Spike 0.25 ml of the 0.20 µg/ml working solution into an empty 250 ml round-bottom flask and allow the acetone to evaporate by placing the flask under a vacuum hood and air drying with the hood on pulling the sash down before adding samples.

Store all standards and matrix spiking solutions at <0°C.

6.0 Procedures

Calibration of equipment

Initial calibration

Prepare an initial five point calibration by analyzing 2 µl each of the working standard concentrations specified in Section 5.0. Calibrate according to the same conditions prescribed in Appendix A. Construct calibration curves using first order or quadratic fit using the five standards for each analyte. Select the fit, which introduces the least calibration error into the quantitation for each compound.

Tabulate the peak areas against concentration for each compound and surrogate

Calculate the response factor (RF) for each compound using the following equation:

$$Cs/As = RF$$

C_s = concentration of the standard compound
A_s = area of the sample peak

Initial calibration data are acceptable if the correlation coefficient, r, is ≥0.990 for linear and the coefficient of the determination, COD, is ≥ 0.990 for non-linear curves calculated across the working concentration range for each compound.

Continuing calibration
Prior to the analysis of each sample set at the beginning of each run sequence and, at a minimum, every 10 samples thereafter during a series of analyses, analyze and evaluate a midpoint calibration solution containing all the selected compounds to ensure that the GC/FPD performance is in compliance with all established criteria. Alternatively, a new five point calibration may be analyzed at the beginning of each run sequence.

Calculate the response factor of each compound in each subsequent standard analysis.

If the response for any analyte varies from the predicted response by more than ± 20 %, a new calibration curve must be prepared for that analyte or the data must be validated and qualified with a report narrative.

Sample Preparation

Remove the samples from refrigerator and allow to reach ambient temperature.

Measure 250 ml of sample into a graduated cylinder. Label each cylinder with the appropriate sample ID. Check pH with pH paper. Record the volume and pH in the extraction logbook.

Prepare a method blank and matrix blank with each group of samples extracted. A method blank consists of a 250 ml volume of laboratory deionized water. A matrix blank consists of a 250 ml volume of laboratory potable water. Add approximately 0.1 g of sodium thiosulfate using a small stainless steel scoop and stir until dissolved.

For each sample selected for matrix spike and matrix spike duplicate analysis, measure out two additional 250 ml aliquots. Prepare matrix spikes by adding the 0.25 ml (250 µl) of matrix spike solution to a 250 ml boiling flask using a microsyringe. Record the amount and lot number of the matrix spike solution in the extraction logbook. Evaporate the residue by placing the flasks under a vacuum hood and air drying with the hood on and the sash down.

Assemble the filter apparatus EM-02 using AC-2 cartridges and the 75 ml reservoir.

Condition the cartridge by sequentially eluting approximately 5 ml of acetone, 10 ml of deionized water, 20 ml of 1 N HCl, and 10 ml of deionized water. DO NOT ALLOW THE CARTRIDGE TO GO DRY AT ANY POINT.

Connect the tubing from the peristaltic pumps to the bottom of the 15 ml reservoirs and fill the reservoirs with approximately 10 ml of DI water. After the conditioning step transfer the filters carefully to the extraction apparatus. Cover the top of the 15 ml reservoir with an index finger and with the other hand pull off the tubing and push the cartridge firmly onto the bottom of the reservoir. Replace tubing onto the bottom of the cartridge.

Snap on the tops to the 15 ml reservoirs tightly with the Teflon tubing securely attached.

Add the samples designated for matrix spikes to the 250 ml flasks to dissolve the residue. Swirl the flasks several times to dissolve the residue. Transfer the sample identification tape to the flasks.

Add 250 µl of tributylphosphate surrogate solution to all samples including blanks and matrix spikes.

Place a teflon line from the extraction apparatus into each graduated cylinder and round bottom flask, ensuring that the line goes to the bottom of the cylinders and flasks. Turn on the peristaltic pump. The peristaltic pump has been previously calibrated to provide a flow rate through the extraction cartridges of approximately 2-3 ml per minute. Verify the

approximate flow rate by setting a timer and measuring the volume removed from each sample after a period of time, between 1-1.5 hours. If the flow rate is higher than 3.5 ml, the peristaltic pump should be recalibrated prior to further extractions.

After the sample has eluted, rinse the container with 10 ml of deionized water and add to the reservoir. Transfer the sample identification tape from the graduated cylinders and round bottom flasks to the cartridges.

Allow the cartridge to run dry for 2 min.

Remove the cartridge, invert it and connect it to a 10 ml Hamilton (or equivalent) glass syringe with a luer adapter using a short piece of Teflon tubing. Transfer the tape with the sample ID onto the neck of the 50 ml pear shaped flask to which that cartridge will be associated with.

Add 10 ml of acetone to the syringe and elute 3 ml in the opposite direction of the sample flow into a 50 ml pear shaped flask.

Stop the elution and allow the cartridge packing material to soak with acetone for 15 minutes before eluting the remaining volume of acetone. Elute the remaining acetone.

Repeat the elution step with an additional 10 ml aliquot of acetone.

Add 8 ml of ethyl acetate to the eluate and evaporate to dryness on a the Bucchi rotary evaporator. If residual water is present, add an additional 5 ml of ethyl acetate and 10 ml of acetone and re-evaporate.

Add 0.5 ml (500 µl) of acetone to the flasks to dissolve the residue. Spike 5 µl of internal standard solution into sample. Swirl the flask to dissolve the residue. Transfer extract to an autosampler vial for analysis.

Label autosampler vials with AC2, the sample ID and the date extracted.

Sample Analysis

These are recommended parameters for the Rtx-200 column. These parameters may be adjusted to optimize responses as necessary.

Due to the addition of co-extractives in the chromatographic system during a series of runs and after performing maintenance on the GC inlet, active sites are formed in the GC system, which result in a reduced response to the pesticide compounds.

Consequently, it is necessary to prime the GC system prior to injection of calibration standards. This is accomplished by injecting five (5) 2 µl aliquots of the 0.300 µg/ml calibration standard just prior to running the calibration curve.

GC and Detector Conditions for analysis of acephate, methamidophos and tributylphosphate.

Method 032900 – Appendix A
Initial oven temperature -150 °C
Initial time - 1 minute
Injection volume - 2 µl
Injector temperature 230 °C
Rate - 25°C/min
Final temperature - 260°C
Final time – 2.50 minutes
Total runtime – 8.90 minutes

7.0 Calculation of Results

The software will calculate the solution concentration in ng/µl injected. The concentration of the sample can be calculated manually by

$$C = (RF_c)\ (A_c)(2)$$

C = Concentration in the sample in µg/L
A_c = Area of the selected compound
RF_c = relative response factor for the selected compound

Sample results are reported to 3 significant figures. For rounding significant figures, refer to EASI SOP *GE-06.01: Reporting Data as a Final Result.*

8.0 QC Requirements

The data files should be quantitated and the instrument run log should be filled in as soon as possible after the analysis is complete. During a batch sequence, the data files are to be queued for quantitation immediately after analysis, and the run log filled in as the sequence is completed.

Gas chromatographic retention times may not shift more than thirty seconds. If this should occur, corrective action may be necessary. Check for system malfunction.

Check for saturation of peaks above the calibration range. Dilute the extract

accordingly and reanalyze.

Calculate the percent surrogate recovery for the surrogate compound. Surrogates are used by the laboratory to facilitate extraction efficiency evaluation only and no criteria have been established.

The maximum holding time before initial sample extraction is 7 days at 4±2 °C. The maximum holding time for final extracts should be 40 days at 0-4 °C.

Method blanks are prepared from deionized water. Matrix blanks are prepared from laboratory potable water. One method blank and one matrix blank is required for every group of 20 samples or each time a group of samples are extracted by the same method whichever is more frequent.

A method blank may not contain more than ½ the PQL for any target compound. When a blank exceeds these limits it is considered to be out of control and the blank and all associated samples must be reextracted or the data must be qualified with a report narrative. The analyst must locate the source of contamination and corrective actions must be taken before data analysis can be continued.

A matrix spike and duplicate are analyzed in order to evaluate the matrix effect of the sample analysis. Matrix spikes and duplicates must be prepared and analyzed each time a group of samples are extracted. Fortified matrix recoveries and relative percent differences are calculated. Matrix recoveries should be between 70 and 120%. The limit for the relative percent difference between spike and duplicate is 40%.

The retention time of the GC peak for the selected compound of interest needs to be within ± 6 seconds of the average retention time for each compound as determined from the initial calibration. When identifying target analytes in a study sample, the peak shape and width will be evaluated manually by visual inspection of the peak to determine that they are similar to those in the fortified samples.

Initial calibration data are acceptable if the correlation coefficient, r, is ≥0.99 for linear and the coefficient of the determination, COD, is ≥ 0.99 for non-linear curves calculated across the working concentration range for each compound or surrogate.

Non-compliance: Analytical performance criteria stated in this SOP may not always be achievable in study samples even when corrective actions were employed in an attempt to meet SOP requirements. In certain pressing situations such as holding time near expiring or quick turnaround requirements, it may be necessary to sacrifice some criteria and proceed with the analysis. Such a decision is left to the study director and will be reported to the study director or his designate as soon as possible. All deviations

will be reported to the study director or his designate as soon as possible. All deviations from the SOP must be thoroughly documented and reported to the study director. The study director is the only individual who can approve changes to the study and will direct the issuance of a protocol deviation.

9.0 Safety

Standard laboratory safety precautions should be adhered to at all times. This assumes that all samples are hazardous.

The use of hoods, safety glasses, lab coats, and any other appropriate safety gear is necessary.

MSDSs are available for all chemicals used in this procedure and should be referred to by all analysts.

Appendix A

GC/FPD Operating Parameters

RUN PARAMETERS

ZERO	=	0
ATT2^	=	4
CHT SP	=	0.6
AR REJ	=	2
THRSH	=	3
PK WD	=	0.04

TIMETABLE EVENTS
EMPTY

CALIBRATION
NO CALIB TBL

INTEGRATION PLOT TYPE	FILTERED
Presentation plot	NO

RUN DATA STORAGE

Store signal data	YES
Device	H
Bunched or raw data	BUNCHED
Local run-time storage	YES
Device	M
Keep run-time storage	NO
Store processed peaks	YES
Device	H

REPORT OPTIONS

Suppress local report	NO
HEIGHT% report	NO
Report uncalibrated peaks	NO
Extended report	NO

PRINT & POST-RUN LIST OPTIONS

Large font	YES
Store post-run report	YES
Device	H
External post-run report	NO
List run parameters	NO
List timetable	NO
List calibration table	NO
List remote method	NO
Form-feed before report	NO
Form-feed after report	NO
Skip perforations in report	NO
Skip perforations in plot	NO

HP 5890A GAS CHROMATOGRAPH
LOOP ADDRESS: 8

OVEN TEMP = 150 SETPT = 150
EQUIB TIME = 0.50 CRYO OFF
OVEN MAXIMUM = 260 CRYO BLAST OFF
INITIAL TEMP = 150
INITIAL TIME = 1.00

```
TEMP PRGM:  RATE          FINAL TEMP          FINAL TIME
                25.0          260                 1.00
 RAMP A         25.0          260                 2.50

RUN LENGTH  =  8.90 MIN

INJ A TEMP  =  230          SETPT  =  230
INJ B TEMP  =  67           SETPT  =  225   <OFF>

RUN LENGTH  =  8.90 MIN

INJ A TEMP  =  230          SETPT  =  230
INJ B TEMP  =  67           SETPT  =  225   <OFF>
DET A TEMP  =  109          SETPT  =  300   <OFF>
DET B TEMP  =  260          SETPT  =  260

SIGNAL 1  =  B
 INET FULL RANGE DATA ON
 RANGE  =  0
 ZERO  =  256.3
 ATTN  =  0

SIGNAL 2  =  B
 INET FULL RANGE DATA ON
 RANGE  =  0
 ZERO  =  0.0
 ATTN  =  0

DETECTOR A  =  FID   <ON>
DETECTOR B  =  FPD   <ON>

PURGE A  =  ON
 ON TIME  =  0.60           OFF TIME  =  0.00
PURGE B  =  ON
 ON TIME  =  0.60           OFF TIME  =  0.00

VALUE 1  =  OFF
VALUE 2  =  OFF

-  TIME TABLE IS EMPTY  -
```

Calibration Report

Data File Name : C:\PEAK\EXPORT1\GC300487.D
Operator :
Instrument : HP5890A
Sample Name :
Run Time Bar Code:
Acquired on : MAR 28, 2000 20:31:43
Report Created on: 30 Mar 00 03:03 PM
Last Recalib on : 30 Mar 00 02:11 PM
Multiplier : 1

Page Number : 1
Vial Number : 0
Injection Number :
Sequence Line :
Instrument Method:
Analysis Method : P033000.MTH
Sample Amount : 0
ISTD Amount :

Calibration Table

Pk#	RT	Lvl	pg/ul	Amt/Area	Ref Istd I#	Name
1	2.400	1	300.0	3.2616e-004	1	methamidophos
		2	200.0	3.518e-004		
		3	100.0	3.6644e-004		
		4	50.0	4.9632e-004		
		5	25.0	4.2462e-004		
2	3.720	1	300.0	9.0746e-004	1	acephate
		2	200.0	9.1858e-004		
		3	100.0	1.0212e-003		
		4	50.0	1.4836e-003		
		5	25.0	1.003e-003		
3	3.975	1	300.0	2.6682e-004	1	tbp
		2	200.0	2.7338e-004		
		3	100.0	2.6366e-004		
		4	50.0	2.6176e-004		
		5	25.0	2.1742e-004		

Calibration Settings

Title:
Five point calibration curve for Acephate/Methamidophos

Reference window: 0.100 minutes
Non-reference window: 0.100 minutes
Units of amount: pg/ul
Multiplier: 1.0
RF uncal peaks: 0.0
ISTD# to adjust uncal peaks: 0
Sample Amount: 0.0

Sample ISTD Information

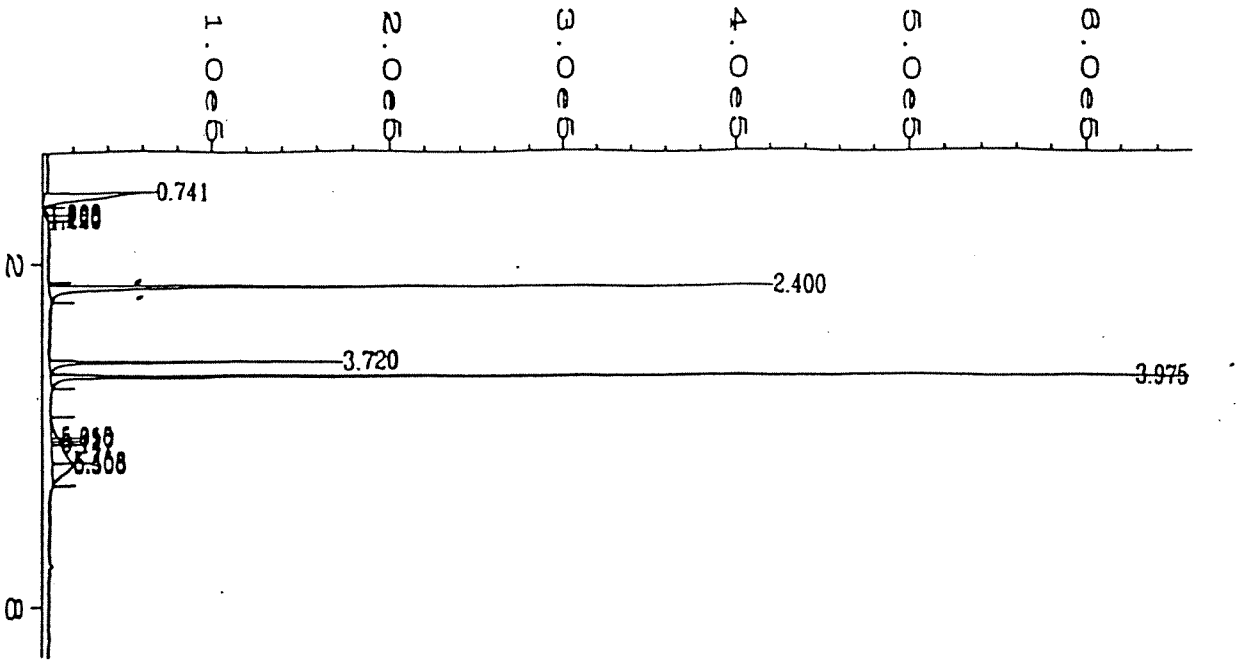
No Sample ISTD Amounts

Multilevel Information

Fit: Linear
Origin: Force

Appendix B

Chromatograms



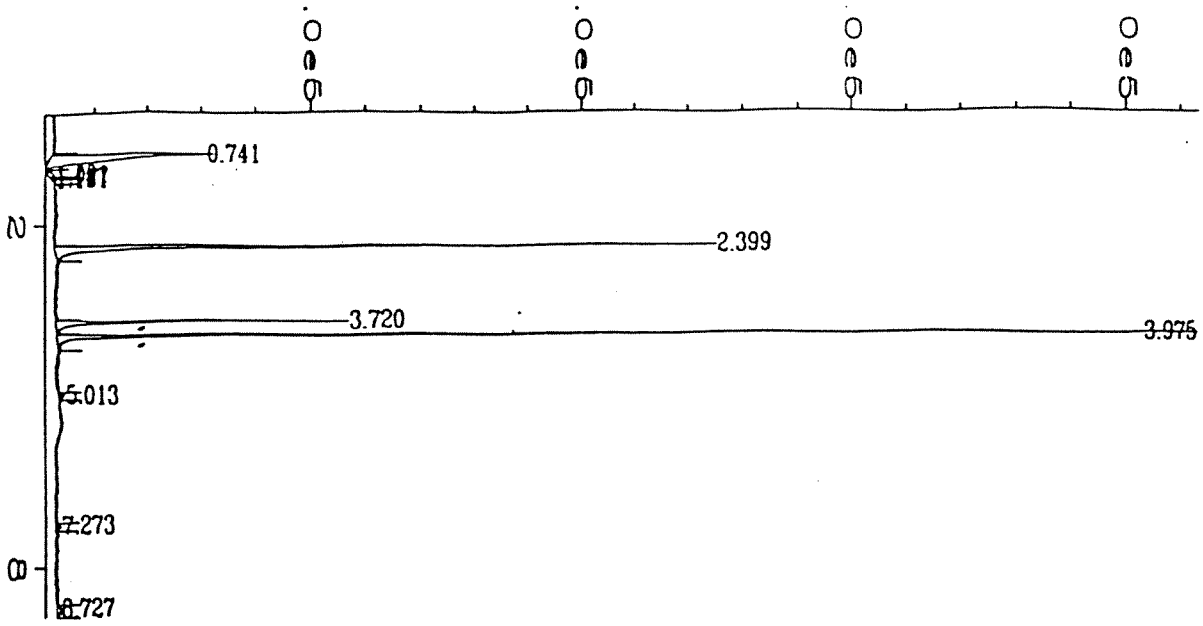
External Standard Report

Data File Name : C:\PEAK\EXPORT1\GC300487.D
Operator :
Instrument : HP5890A
Sample Name :
Run Time Bar Code:
Acquired on : MAR 28, 2000 20:31:43
Report Created on: 30 Mar 00 02:14 PM
Last Recalib on : 30 Mar 00 02:11 PM
Multiplier : 1

Page Number : 1
Vial Number : 0
Injection Number :
Sequence Line :
Instrument Method:
Analysis Method : P033000.MTH
Sample Amount : 0
ISTD Amount :

Sig. 1 in C:\PEAK\EXPORT1\GC300487.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
2.400	919801	PV	0.033	1	304.703	methamidophos
3.720	330593	PV	0.029	1	300.255	acephate
3.975	1124343	VV	0.026	1	302.814	tbp



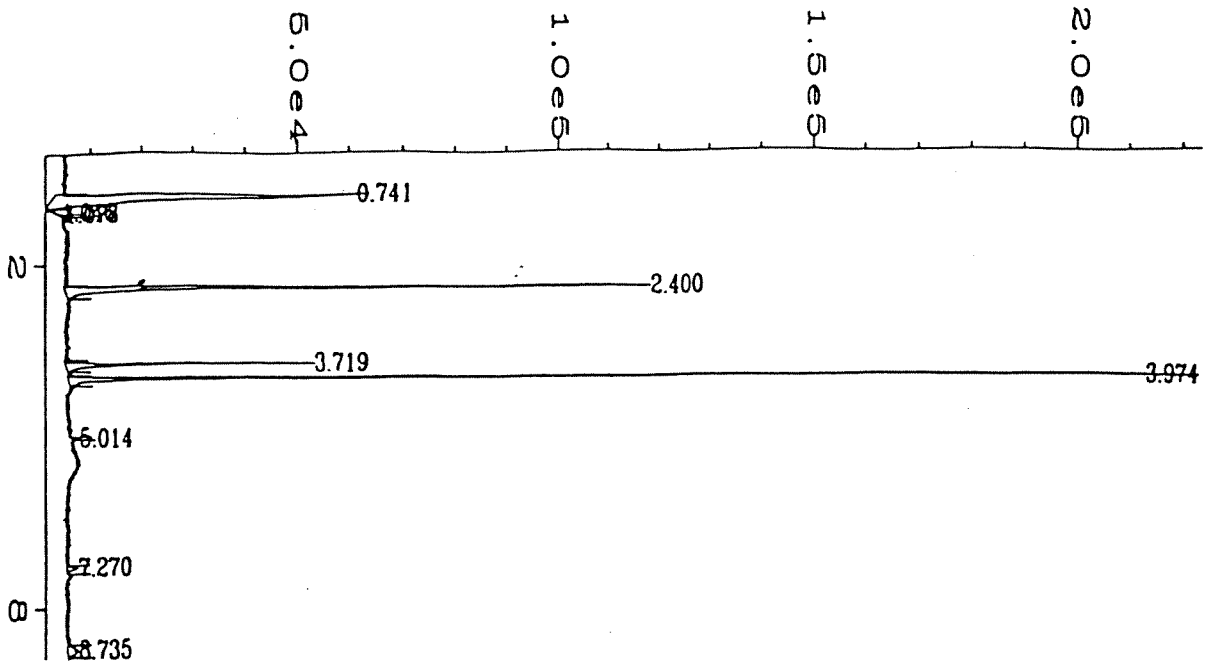
External Standard Report

Data File Name : C:\PEAK\EXPORT1\GC300488.D
Operator :
Instrument : HP5890A
Sample Name :
Run Time Bar Code:
Acquired on : MAR 28, 2000 20:43:44
Report Created on: 30 Mar 00 02:15 PM
Last Recalib on : 30 Mar 00 02:11 PM
Multiplier : 1

Page Number : 1
Vial Number : 0
Injection Number :
Sequence Line :
Instrument Method:
Analysis Method : P033000.MTH
Sample Amount : 0
ISTD Amount :

Sig. 1 in C:\PEAK\EXPORT1\GC300488.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
2.399	568498	PV	0.034	1	193.402	methamidophos
3.720	217727	VV	0.030	1	201.891	acephate
3.975	731577	VV	0.027	1	195.833	tbp



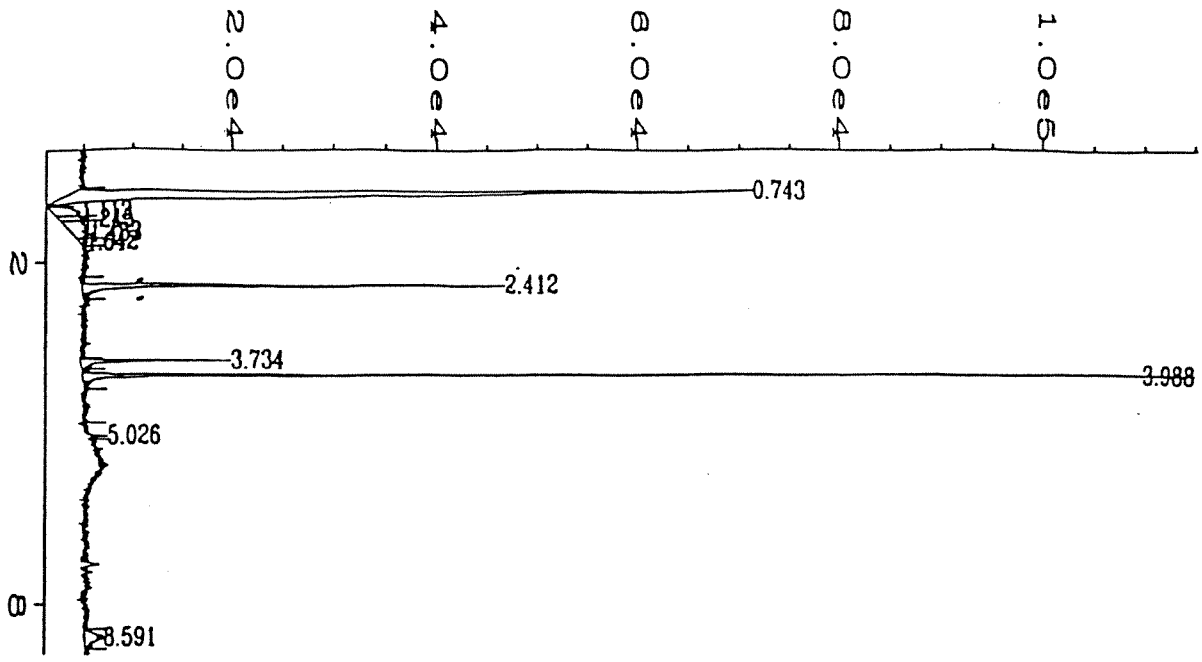
External Standard Report

Data File Name : C:\PEAK\EXPORT1\GC300489.D
Operator :
Instrument : HP5890A
Sample Name :
Run Time Bar Code:
Acquired on : MAR 28, 2000 20:55:42
Report Created on: 30 Mar 00 02:16 PM
Last Recalib on : 30 Mar 00 02:11 PM
Multiplier : 1

Page Number : 1
Vial Number : 0
Injection Number :
Sequence Line :
Instrument Method:
Analysis Method : P033000.MTF
Sample Amount : 0
ISTD Amount :

Sig. 1 in C:\PEAK\EXPORT1\GC300489.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
2.400	272900	PV	0.035	1	99.749	methamidophos
3.719	97927	PV	0.030	1	97.483	acephate
3.974	379278	BV	0.027	1	99.874	tbp



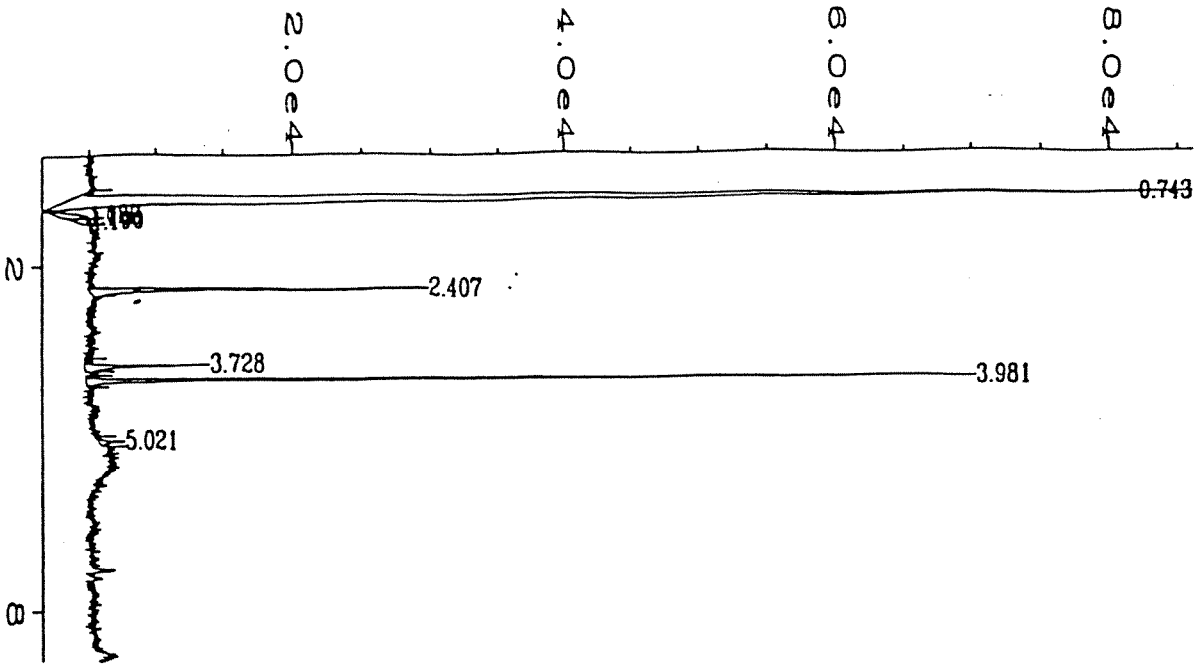
External Standard Report

Data File Name : C:\PEAK\EXPORT1\GC300490.D
Operator :
Instrument : HP5890A
Sample Name :
Run Time Bar Code:
Acquired on : MAR 28, 2000 22:11:54
Report Created on: 30 Mar 00 02:16 PM
Last Recalib on : 30 Mar 00 02:11 PM
Multiplier : 1

Page Number : 1
Vial Number : 0
Injection Number :
Sequence Line :
Instrument Method:
Analysis Method : P033000.MTH
Sample Amount : 0
ISTD Amount :

Sig. 1 in C:\PEAK\EXPORT1\GC300490.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
2.412	100742	BB	0.062	1	45.205	methamidophos
3.734	33701	PV	0.047	1	41.509	acephate
3.988	191014	VV	0.028	1	48.594	tbp



External Standard Report

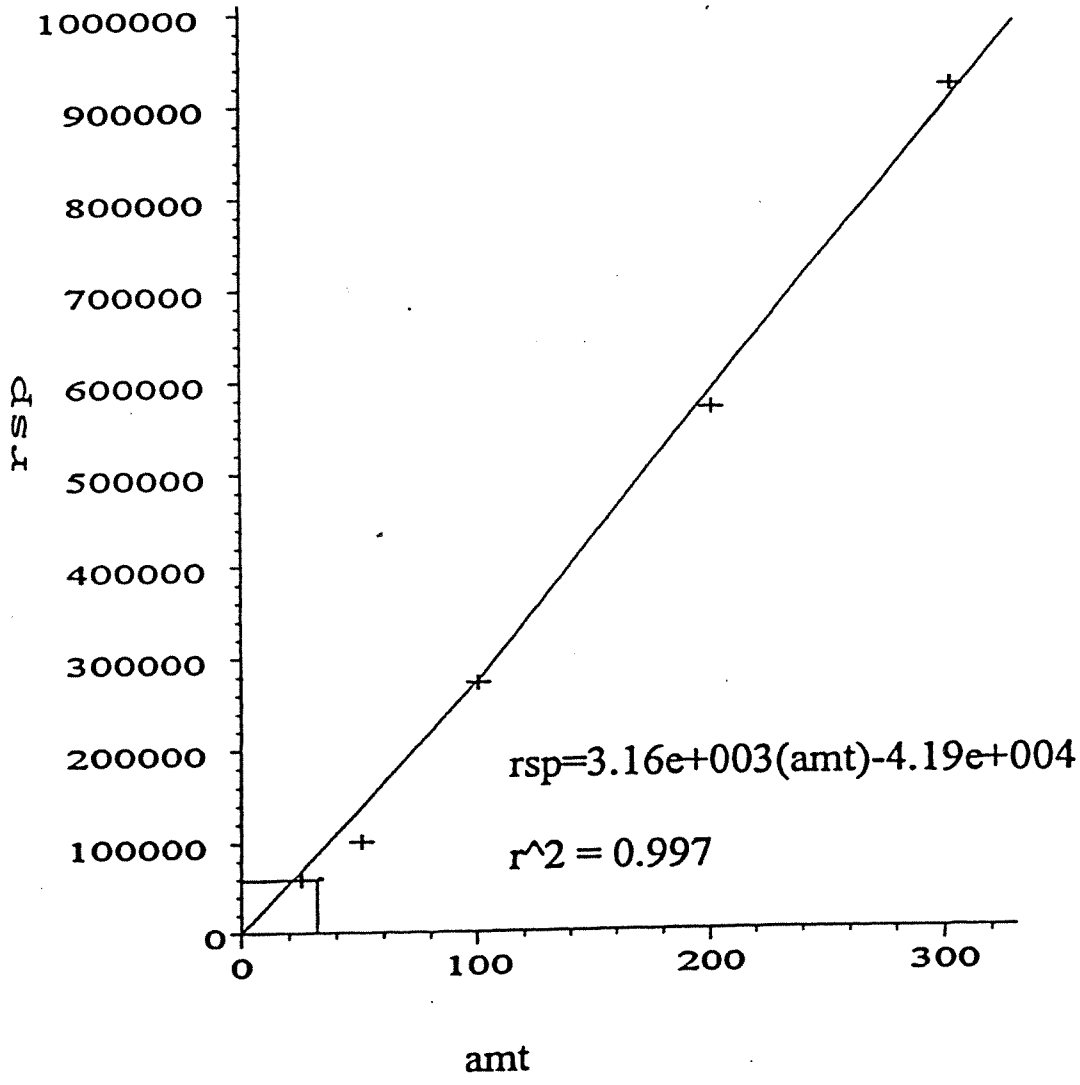
Data File Name : C:\PEAK\EXPORT1\GC300491.D
Operator :
Instrument : HP5890A
Sample Name :
Run Time Bar Code:
Acquired on : MAR 28, 2000 22:23:56
Report Created on: 30 Mar 00 02:16 PM
Last Recalib on : 30 Mar 00 02:11 PM
Multiplier : 1

Page Number : 1
Vial Number : 0
Injection Number :
Sequence Line :
Instrument Method:
Analysis Method : P033000.MTH
Sample Amount : 0
ISTD Amount :

Sig. 1 in C:\PEAK\EXPORT1\GC300491.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
2.407	58876	PV	0.035	1	31.941	methamidophos
3.728	24926	PV	0.037	1	33.862	acephate
3.981	114985	VV	0.027	1	27.885	tbp

methamidophos



PROTOCOL AMENDMENT FORM

AMENDMENT NUMBER: 3

COPY

Protocol: En-fate Study No. 00102

Protocol Title: CHLORINE DEGRADATION OF SELECTED ORGANOPHOSPHORUS PESTICIDES AND CERTAIN OF THEIR DEGRADATES IN A DRINKING WATER MATRIX

Compound/Formulation: Acephate, Azinphos-methyl, Chlorpyrifos, Diazinon, Malathion, Methamidophos and major degradation products.

AMENDMENT(S):

1) TITLE

CHANGES: CHLORINE DEGRADATION OF SIX ORGANOPHOSPHORUS INSECTICIDES AND FOUR OXONS IN A DRINKING WATER MATRIX.

REASON(S): New project title is more descriptive of the study performed.

Effect of change: Provides a clearer description of the project.

Effective date of this Amendment: January 30, 2001

STUDY DIRECTOR: _____ DATE 2/5/2001

Amendments to be distributed per ~~Protocol~~ Distribution List

Environmental Analytical Solutions, Inc.

PROTOCOL DEVIATION

COPY

STUDY NUMBER: En-Fate Study No. 00102
TITLE OF STUDY: Community Water System Surface Drinking Water Monitoring Study for Organophosphate Pesticides and Their Major Degradation Products in the United States
STUDY DIRECTOR: Dennis P. Tierney, Ph.D.
PROTOCOL DEVIATION: 00102-01
DEVIATION RELATING TO:

[ ] Facilities
[ ] Test systems
[ ] Test material
[ ] Test dates
[ ] Dosage and preparation
[ ] Test procedures
[ ] Support areas
[X] Other

PROTOCOL INFO: SOP No. EASI MS-20.03, Section 8.0: The maximum holding time for extracts should be 40 days at 0-4°C.

DEVIATION: Some extracts have been analyzed after the recommended 40-day holding time.

EXPLANATION: Problems with matrix-specific effects were observed in C-18 extracts for azinphos methyl and azinphos methyl oxon and AC-2 extracts for acephate and methamidophos. This required extensive method development that delayed sample analysis of some extracts. Extraction holding times were not affected.

EFFECT ON STUDY: Comparison of average target analyte recovery in matrix spike/matrix spike duplicate extracts analyzed within 40 days of extraction and after 40 days of extraction was performed. Results confirmed the stability of the target organophosphorus pesticides and their primary degradants in extracts exceeding the recommended extract holding time of 40 days. Subsequent to the completion of all sample analyses, comparisons of average recoveries of target analytes from extracts analyzed within 40 days and after 40 days of extraction will be attached to document extract stability.

APPROVAL:

Performing Laboratory Laboratory Coordinator _____ Date 8-14-99

Study Coordinator _____ Date 8-17-99

Study Director _____ Date 8-24-99

Comparison of Analyte Matrix Spike Recoveries for Extracts Analyzed Within and Exceeding 40 Days Storage

Compounds	Extracts Less Than 40 Day Storage Average % Recovery	Extracts Exceeding 40 Day Storage Average % Recovery
Azinphos Methyl	96	107
Azinphos Methyl Oxon	115	165
Diazinon	91	98
Diazinon Oxon	96	109
Malathion	93	101
Malathion Oxon	93	103
Chlorpyrifos	93	103
Chlorpyrifos Oxon	92	98
Acephate	84	98
Methamidophos	70	80

Extracts Exceeding 40 Days Storage:

Range of Days Between Extraction and Analysis

C-18 Extracts (Azinphos Methyl, Chlorpyrifos, Diazinon, Malathion and Oxons)	41-202 days
AC-2 Extracts (Acephate, Methamidophos)	41-332 days

Average Days Between Extraction and Analytis

C-18 Extracts (Azinphos Methyl, Chlorpyrifos, Diazinon, Malathion and Oxons)	125 days
AC-2 Extracts (Acephate, Methamidophos)	187 days